VOLUME XXXV SEPTEMBER, 1956 NUMBER 9

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

Contents

The Effect in Humans of Extracellular pH Change on the Relationship between Serum Potassium Concentration and Intracellular Potassium. JAMES M. BURNELL, MARIO F. VILLAMIL, BEN T. UYENO, AND BELDING H. SCRIBNER 935

Prolonged Hypothermia in Experimental Pneumococcal Peritonitis. B. EISEMAN, W. G. MALETTE, R. S. WOTKYNS, W. B. SUMMERS, AND J. L. TONG 940

The Urinary Excretion of Sodium and Potassium During the Pyrogenic Reaction in Man. WILLOUGHBY LATHEM 947

Studies on the Physiology of Hunger. I. The Effect of Intravenous Administration of Glucose on Gastric Hunger Contractions in Man. ALBERT J. STUNKARD AND HAROLD G. WOLFF 954

Determination of the Life Span of Human Blood Platelets Using Labelled Diisopropylfluorophosphonate. C. H. W. LEEKSMA AND J. A. COHEN 964

The Relationship of Cardiovascular and Renal Hemodynamic Function to Sodium Excretion in Patients With Severe Heart Disease but Without Edema WILLIAM HOLLANDER AND WALTER E. JUDSON .. 970

A Study of Serum Lipoproteins in Normal and Atherosclerotic Patients by Paper Electrophoretic Techniques. WILLIAM P. JENCKS, MARION R. HYATT, MERA R. JETTON, THOMAS W. MATTINGLY, AND E. L. DURRUM 980

Carbohydrate Metabolism in Intact Skeletal Muscle in Man During the Night. KENNETH L. ZIERLER AND REUBIN ANDRES 991

Hyposthenuria in Sickle Cell Anemia: A Reversible Renal Defect. H. G. KEITEL, D. THOMPSON, AND H. A. ITANO 998

The Diffusing Capacity of the Lungs in Patients with Mitral Stenosis Studied Post-Operatively. R. L. RILEY, C. J. JOHNS, G. COHEN, J. E. COHN, D. G. CARROLL, AND R. H. SHEPARD 1008

An Evaluation of Potassium Iodide as a Therapeutic Agent in the Treatment of Experimental Hypercholesteremia and Atherosclerosis. MEYER FRIEDMAN, RICHARD HOMER, AND SANFORD O. BYERS ... 1015

The Metabolism of Chylomicra. I. The Removal of Palmitic Acid-1-C^{14} Labeled Chylomicra from Dog Plasma. RICHARD J. HAVEL AND DONALD S. FREDRICKSON 1025

Tracer Studies of the Absorption and Fate of Steroid Hormones in Man. LEON HELLMAN, H. LEON BRADLOW, E. L. FRAZELL, AND T. F. GALLAGHER 1033

The Effects of Compound A (11-Dehydrocorticosterone) and Compound F (17-Hydroxycorticosterone) on Total Body Fat. LAURENCE H. KYLE, RICHARD J. MEYER, MARCUS SCHAAF, AND EDWARD J. WERDEIN 1045

Plasma Regression, Distribution and Excretion of Radiomercury in Relation to Diuresis Following the Intravenous Administration of Hg^{203} Labelled Chlormerodrin to the Dog. R. R. M. BORGHGRAEF, R. H. KESSLER, AND R. F. PITTS 1055

The Journal of Clinical Investigation

Printed by the Lancaster Press, Inc., Prince and Lemon Sts., Lancaster, Pa. Published by the American Society for Clinical Investigation. Publication Office, 622 West 168th St., New York 32, N. Y.

STANLEY E. BRADLEY, EDITOR-IN-CHIEF

ASSOCIATE EDITORS

LUDWIG W. EICHNA IRVING M. LONDON

The Editorial Committee of the American Society for Clinical Investigation

THOMAS P. ALMY, *New York, N. Y.*
ROBERT W. BERLINER, *Bethesda, Md.*
PHILIP K. BONDY, *New Haven, Conn.*
FRANCIS P. CHINARD, *Baltimore, Md.*
GEORGE L. CURRAN, *Kansas City, Kan.*
LEWIS DEXTER, *Boston, Mass.*
DAVID P. EARLE, JR., *Chicago, Ill.*
RICHARD V. EBERT, *Little Rock, Ark.*
LEONARD P. ELIEL, *Oklahoma City, Okla.*
WARD S. FOWLER, *Rochester, Minn.*
CHARLES H. RAMMELKAMP, *Cleveland, O.*
EDWARD C. REIFENSTEIN, JR., *Butler, N. J.*
I. HERBERT SCHEINBERG, *New York, N. Y.*
RICHARD W. VILTER, *Cincinnati, O.*
LAWRENCE E. YOUNG, *Rochester, N. Y.*

THE JOURNAL OF CLINICAL INVESTIGATION is designed for the publication of original investigations dealing with or bearing on the problems of disease in man, and it is the policy of the editors that the JOURNAL should cover the field of clinical investigation in its broadest sense.

Contributions should be sent to the **Editor-in-Chief, Dr. Stanley E. Bradley, 622 West 168th St., New York 32, N. Y.** In order to hasten the process of reviewing and editing it is requested that each original manuscript be accompanied by a duplicate copy and duplicate illustrations.

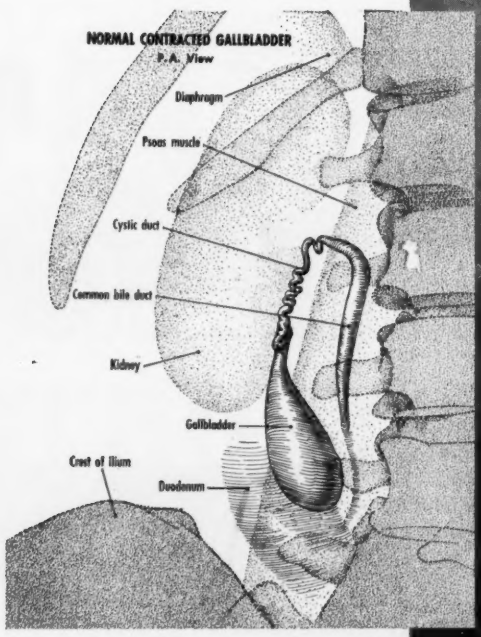
In order further to expedite the earliest possible publication of articles submitted to the JOURNAL OF CLINICAL INVESTIGATION they will be published in the order of final processing after review, correcting and editing rather than in the strict sequence of their receipt in this office. Papers which require much editorial change, revision of charts and tables, condensation and rewriting will be delayed according to the time required for their correction. This policy will put a premium on excellence of manuscript as well as the major consideration of content. Because of the increasing costs of printing, especially in the hand work of making tables, the editors suggest that when feasible photographs of tables be prepared. It is also requested that charts be photographed, the photographs accompanying the manuscript. The originals of the charts need not be forwarded except by special request of the editors. Further information regarding the preparation of manuscripts may be obtained from the editors.

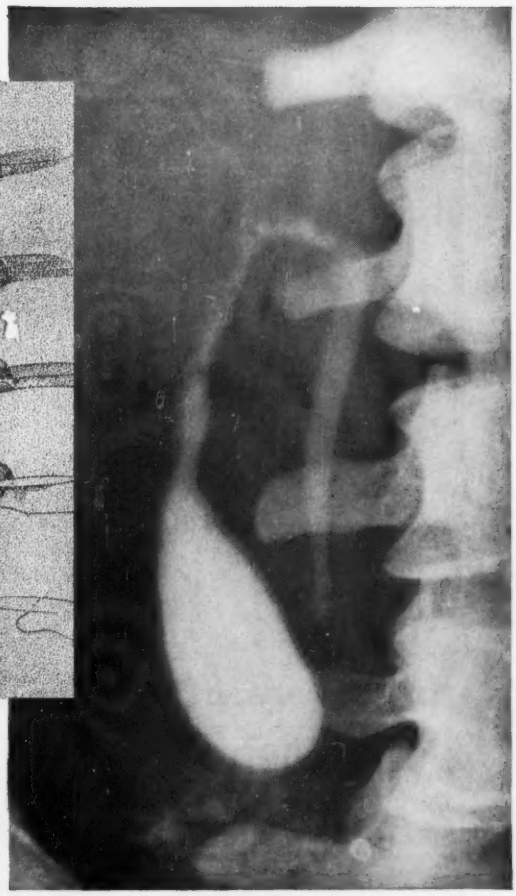
Each volume will consist of 12 monthly issues appearing throughout the calendar year. The subscription price is $12 per volume, **payable in advance;** foreign countries also $12, payable in U. S. funds. Single copies will be sold when available at Vols. 1–28, $2; Vols. 29–34, $1.50 a copy. Authors may purchase reprints at scheduled prices. **A special subscription rate of $6 per volume of 12 monthly issues is offered to medical students, interns, fellows, and residents of approved hospitals.** Subscriptions are **net postpaid.** Remittance should be made by draft, check or postal money order, payable to **The Journal of Clinical Investigation, 622 West 168th St., New York 32, N. Y.** Claims for copies lost in the mails must be received within **thirty** days (domestic), **ninety** days (foreign), of the date of issue. Changes of address must be received at least two weeks in advance of issue.

Acceptance for mailing at special rate of postage provided for in paragraph (d-2), section 34.40, P.L. & R. of 1948, authorized February 13, 1950

Entered as second-class matter September 16, 1931, at the Post Office at Lancaster, Pa., under the Act of March 3, 1879

Copyright © 1956, American Society for Clinical Investigation





"There are three main advantages in the use of

Telepaque®

SUPERIOR ORAL CHOLECYSTOGRAPHY AND CHOLANGIOGRAPHY

1. **Excellent cholecystograms are readily obtainable.**
2. **The side reactions are usually minimal, only rarely very disturbing, and often completely absent.**
3. **In a fairly large percentage of cases, the cystic and the common ducts are quite definitely outlined, and occasionally even the hepatic duct."**

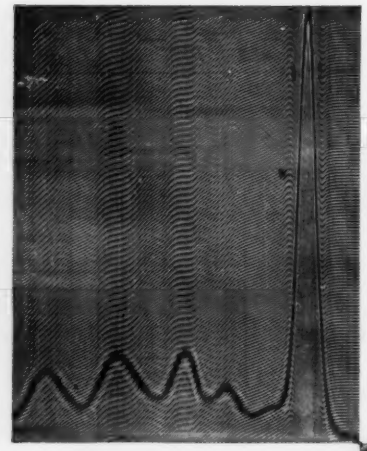
Buckstein, Jacob: The Digestive Tract in Roentgenology. Philadelphia, J. B. Lippincott Co., 2nd ed., 1953, vol. 2, p. 1003.

DOSAGE:
The average adult dose of Telepaque is 3 Gm. (6 tablets). In persons of thin or medium build, weighing less than 150 lb., 2 Gm. (4 tablets) may be sufficient.

SUPPLIED:
Tablets of 0.5 Gm. in envelopes of 6 tablets, boxes of 5 and 25 envelopes, and bottles of 500.

Winthrop LABORATORIES
NEW YORK 18, N.Y. • WINDSOR, ONT.

Telepaque (brand of iopanoic acid), trademark reg. U.S. Pat. Off.



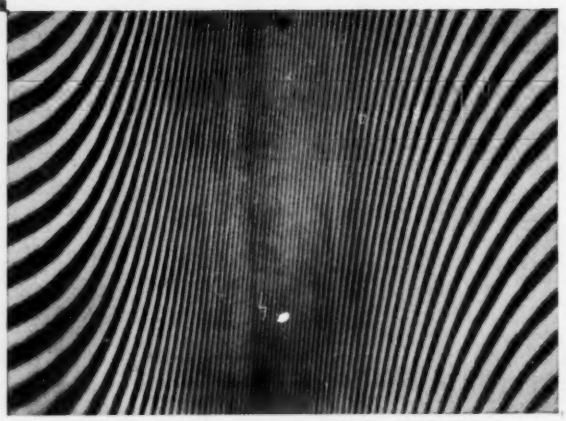
Ascending branch of albumin peak.

Model 38-A Electrophoresis Apparatus.

Now!

for users of Electrophoresis Apparatus

greater speed and precision



Detail of albumin peak shows fine fringe structure.

Now P-E makes available a Rayleigh fringe system attachment to the Model 38 and 38-A Tiselius Electrophoresis instruments. The new accessory makes possible fast, direct measurement of refractive index change—photographically records derivative pattern, base line and full fringe field simultaneously. And permits considerably greater precision than is obtainable with conventional systems.

New also is a compact, efficient refrigeration unit which maintains water bath temperature within ±0.02°C. — eliminating striations or distortions caused by ice bath temperature fluctuations.

The model 38 itself is well known in hospitals and laboratories throughout the world. Here are some of the features of the new Model 38-A:

- **Land Camera**—pictures of patterns ready in 1 minute — slides, duplicates, etc., easily made from original.
- **Top-side viewer** — for convenient observations of pattern formation and ordinary film photography.
- **Cylindrical lens viewing system** — entire pattern is visible to operator in ground glass screen.
- **Sealed schlieren cell** — eliminates fogging and need for dry air pump.
- **New source light assembly** — 15x the intensity of the old.
- **Improved entrance and exit slits** — provide optimum illumination and resolution.
- **Auxiliary exit slit** — permits adjusting range and sensitivity for specific applications.
- **Simplified power supply** — now built into instrument as standard equipment.

Perkin-Elmer CORPORATION

Norwalk, Connecticut

CONFIDENCE

inspired by performance

TERRABON®

BRAND OF OXYTETRACYCLINE
HOMOGENIZED MIXTURE

delightfully peach-flavored, peach-colored, taste-tempting, time-saving liquid oral preparation of Terramycin.

125 mg. oxytetracycline per 5 cc. teaspoonful. Specially homogenized, providing therapeutic blood levels in one hour. Packaged ready to use.

Bottles of 2 fl. oz. and 1 pint.

a new,
ready-to-use
liquid form of

TERRAMYCIN®

BRAND OF OXYTETRACYCLINE

rapidly effective . . . well tolerated . . . consistently dependable in the widest range of clinical infections.

A complete selection of convenient dosage forms for every need in broad-spectrum antibiotic therapy.

Pfizer

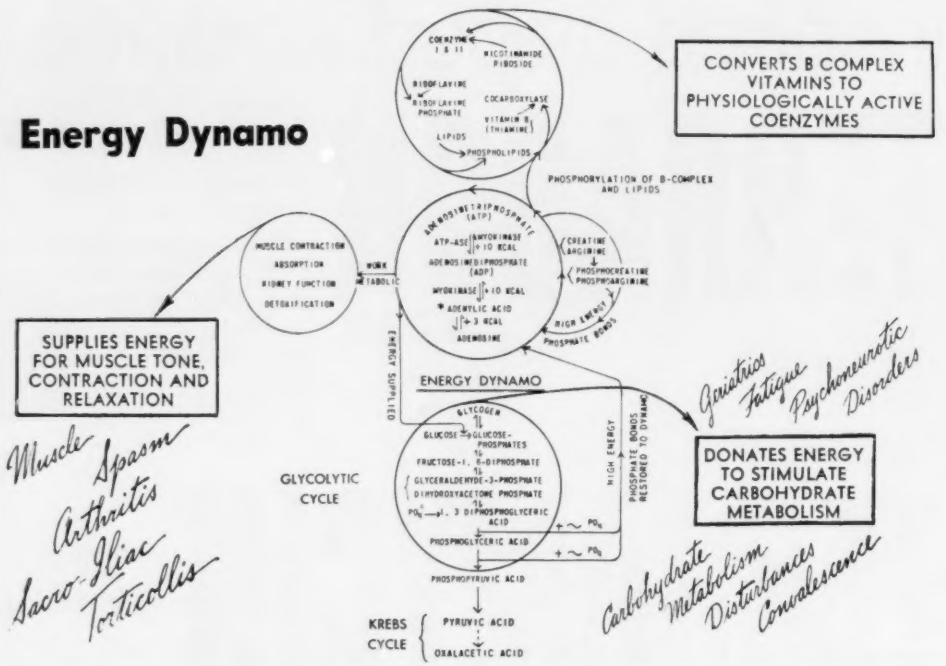
PFIZER LABORATORIES
Division, Chas. Pfizer & Co., Inc.
Brooklyn 6, N. Y.

IRONYL®

The Iron Salt of **Adenosine-3-Monophosphate**

THE ROLE OF THE ADENYLIC SYSTEM IN ENERGY EXCHANGES GOVERNING PHYSIOLOGICAL PROCESSES

Energy Dynamo



Clinically: IRONYL is available in one cc ampuls and sublingual tablets.

Gradually the role of the Adenylic Nucleotides in clinical medicine is being clarified leading to applications in an expanding number of indications, particularly where there are disturbances in the metabolism of muscle energy.

IRONYL is indicated in the treatment of diseases associated with spasm and insufficient relaxation of striated muscles such as arthritis, sacro-iliac, torticollis; in B complex therapy, fatigue and exhaustion.

Ironyl is supplied in boxes of 10, 25, 100 ampuls and 12, 25, and 250 tablets.

PHYSIOLOGICAL CHEMICALS COMPANY, INC.

New Rochelle, N. Y.

PHYSIOLOGICAL CHEMICALS EST. 1935 PC

AVAILABLE ON REQUEST an annotated Bibliography, "ABSTRACTS ON THE ADENYLIC NUCLEOTIDES" with particular reference to Yeast Adenylic Acid (Adenosine-3-Monophosphate).

Modern medical research instruments

THESE seven-ton bulldozers are truly research tools, for they are taking part in an exhaustive program for the study and revision of accepted methods of medical instrument design and manufacture.

Yes, Sanborn Company is on the move! The instruments above are levelling off small mountains of earth and rock in preparation for a new and modern Sanborn plant near Boston, Mass.

Completion of the structure late this year will mean vastly improved facilities for research, manufacturing and other operations. This will directly and immediately benefit not only the work Sanborn does, but also the people who use Sanborn instruments. It will make possible more rapid development of new instruments . . . faster production, delivery and service . . . and increased opportunity for a larger number of people to apply their skills to the problems of modern instrument design and manufacture.

Sanborn Company, Cambridge 39, Massachusetts

Scale model of new Sanborn plant just off Route 128 in Waltham, Mass.

Relax the best way ... pause for Coke

continuous quality
is quality you trust

DRINK
Coca-Cola
REG. U.S. PAT. OFF.

NOW OVER 5100
BIOS CHEMICALS

- Germanochloroform
- α-Glucosethiourea
- Glucovanillin
- Glutaconic Acid
- Glutamylcysteine
- Glutarimide
- Glycolamide
- Glycylglycyl-d-alanine
- Glycyl-l-proline
- Graphitic Acid
- Guaiacol Cacodylate
- Guanidine Base
- Guanylurea Sulfate
- Gulonic Acid
- Gulose, d (+)
- Gymnemic Acid
- Hafnium Oxide
- Hemicellulase
- Hemicellulose
- Heptanediol-1, 7
- Herzynine

Ask for our new complete catalogue

BIOS Laboratories, Inc.
17 West 60th St. New York 23 N. Y.
PLaza 7-8171

The Journal of Clinical Investigation
622 West 168th Street — New York 32, New York

COMPLETE VOLUMES OF BACK ISSUES

Vols. 1 through 11, inc.—special price	$25.00
Vol. 12 (1933) six issues	10.00
Vol. 13 (1934) six issues	10.00
Vol. 27 (1948) six issues and one supplement	10.00
Vol. 28 (1949) six issues and two supplements	10.00
Vol. 29 (1950) twelve issues	10.00
Vol. 30 (1951) twelve issues and one supplement	12.00
Vol. 31 (1952) twelve issues	12.00
Vol. 32 (1953) twelve issues	12.00
Vol. 33 (1954) twelve issues	12.00
Vol. 34 (1955) twelve issues and one supplement	12.00
Index, Vols. 1–17, inc.	2.00

Order While Available

Automation in pharmaceutical research

Automation is now playing an increasingly important role in pharmaceutical research. At Upjohn, a new electronic analog computer has been developed with the hope of facilitating hormone synthesis and the isolation of new antibiotics.

The need for an entirely new type of computer became evident when Upjohn research personnel developed a theoretical method of operating a 20-foot fractionating column which, if proved practical, would make possible the separation of highly complex mixtures. However, some quicker way of checking than either trial-and-error or repetitive arithmetic was needed to evaluate this theoretical operation against orthodox methods of operating the column. The solution was offered by Upjohn physicists, who worked out a relatively simple, inexpensive circuit for a computer that would calculate the problems, not in the adding-subtracting sense, but by forcing electrical energy to behave according to established laws of liquid extraction.

The electronic simulator does its work three to four hundred times as fast as a human with pencil and slide rule; expediency that may bring about an earlier appearance of certain types of new and improved pharmaceuticals.

Upjohn

medicine . . .
designed for health . . .
produced with care

REVUE FRANÇAISE D'ÉTUDES CLINIQUES ET BIOLOGIQUES

SEPTEMBER, 1956

Editorial

Prevention of Accidents Caused by Radiation. M. TUBIANA.

Original Articles

Heparin Inhibition of the Formation of Thrombi. M. BURSTEIN and J. LOEB.

A Study of Luteal Function in the "Premenstrual Syndrome". S. GELLER, G. RUCART, Y. VALLIN, and M. F. JAYLE.

New Determinations of Protein Fractions in the Sera of Patients with Diseases of the Muscles. A. LOWENTHAL and M. VAN SANDE.

Studies of the Muscular Lesions in 46 Cases of Collagen Disease. P. A. MAURICE.

General Review

On the Multiplicity of Structures and Functions of Striated Muscle. A. BOURGUIGNON.

Role of Chemical and Physical Agents in the Pathogenesis of Certain Neoplasms in Man. G. RUDALI.

Technical Note

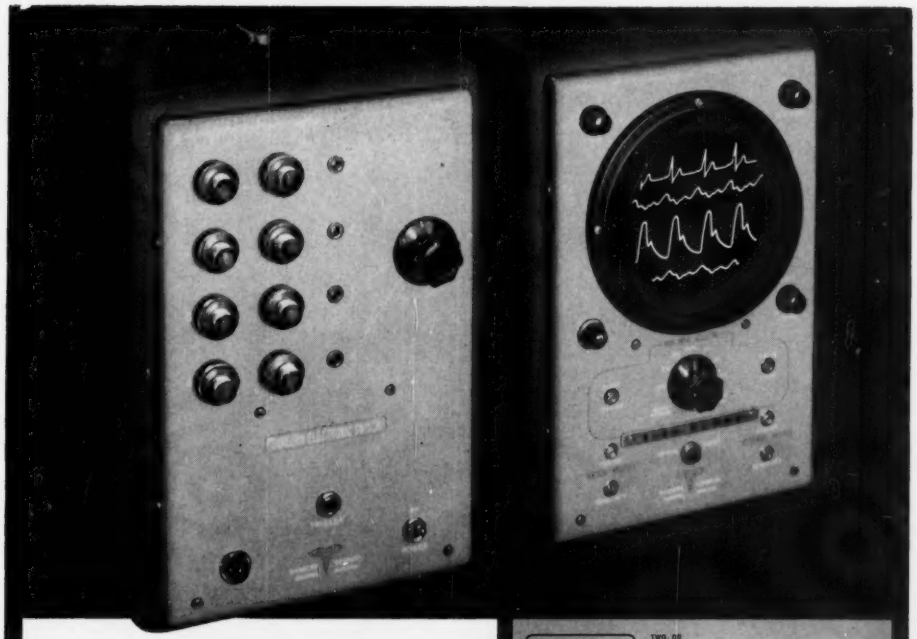
Precision of Methods Employed in the Study of Pulmonary Ventilation. P. SADOUL, P. PILLOT, and J. GRAIMPREY.

* * * * *

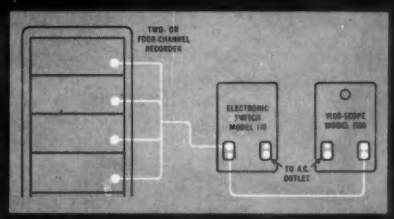
Subscription, U.S.A., and Canada, $19.00

Special rate for interns and residents, $13.00

Flammarion, 1243 University Street, Montreal



For viewing up to four physiological events simultaneously.



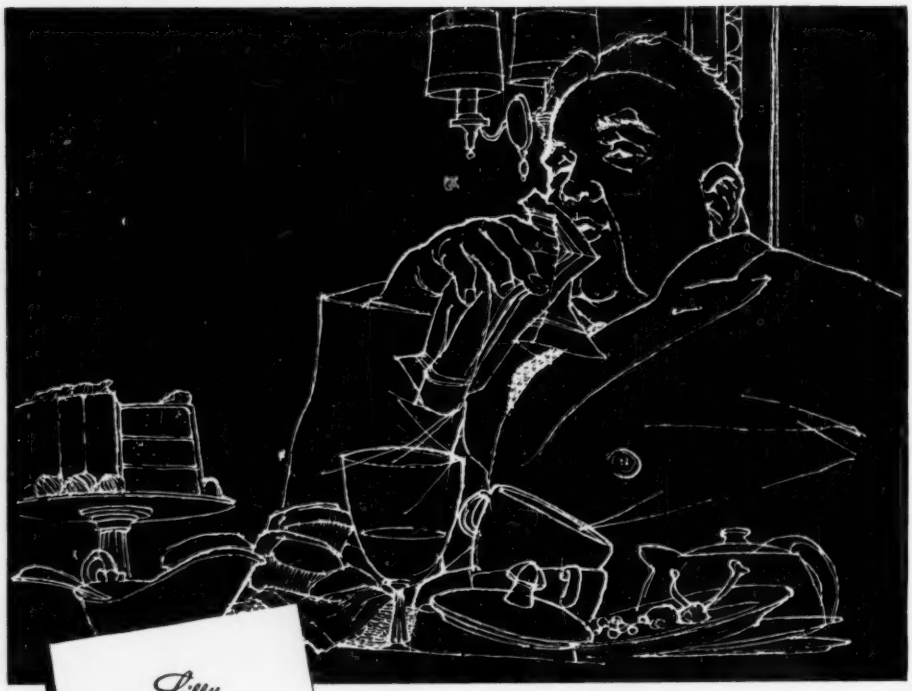
THE **SANBORN** ELECTRONIC SWITCH

The new Model 179 Electronic Switch has been designed for use with the Sanborn Viso-Scope Model 169A and anyone of the Sanborn direct-writing recording systems. With this equipment, the doctor, researcher or teacher can view the waveforms of as many as *four* phenomena simultaneously. A typical example would be the viewing, during recording, of (1) an ECG, (2) carotid artery pressure, (3) liver pulsation, and (4) jugular vein pressure. When less than four traces are desired, the others may be moved off the Viso-Scope screen by means of "Position" controls.

In the design of the Sanborn Electronic Switch, special emphasis has been placed on simplicity of operation. Turning a single knob selects any one input signal, or all signals. Both vertical height and position of signal traces, on Viso-Scope screen, may be controlled individually. Interconnection of recording system, Switch and Viso-Scope is also facilitated by means of plug-in cables furnished with Electronic Switch. A descriptive bulletin which gives complete operating features and price information on this instrument and related Sanborn equipment is available on request.

The Model 179 Electronic Switch and Model 169A Viso-Scope are products of the same designers and makers as the well-known Viso-Cardiette, direct writing electrocardiograph.

SANBORN COMPANY
CAMBRIDGE 39, MASS.



relieves after-eating distress and chronic constipation

'Bilron'

(IRON BILE SALTS, LILLY)

a potent choleretic

Under the priming influence of 'Bilron,' natural bile flow and concentration of bile acids are greatly increased. When symptoms include intolerance to fats, constipation, or flatulence, 'Bilron' offers effective, gratifying relief. Also, 'Bilron' dissolves in the small intestine at the optimal point for emulsification and absorption of fats.

Available in 2 1/2-grain and 5-grain pulvules.

DOSAGE: Usually 5 to 10 grains daily with meals.

80TH ANNIVERSARY 1876 · 1956 / ELI LILLY AND COMPANY

662012

THE EFFECT IN HUMANS OF EXTRACELLULAR pH CHANGE ON THE RELATIONSHIP BETWEEN SERUM POTASSIUM CONCENTRATION AND INTRACELLULAR POTASSIUM [1]

By JAMES M. BURNELL, MARIO F. VILLAMIL,[2] BEN T. UYENO, AND BELDING H. SCRIBNER

(From the Veterans Administration Hospital and the Department of Medicine, University of Washington School of Medicine, Seattle, Wash.)

(Submitted for publication March 20, 1956; accepted May 10, 1956)

For many years investigators have been aware of the association of hyperkalemia with acidosis and hypokalemia with alkalosis. The early *in vitro* work of Fenn and Cobb (1) suggested that there might be a cause and effect relationship. However, it was not until recently that satisfactory *in vivo* data were obtained (2–7) which permitted the formulation of the hypothesis that changes in extracellular pH altered the extracellular potassium concentration independently of changes in total body potassium [3] (7). Thus, in animals, acidosis increases and alkalosis decreases the serum potassium concentration independently of changes in total body potassium. The present study demonstrates similar interrelationships in humans.

EXPERIMENTAL PLAN AND METHODS

Five patients were selected for study because of alterations of extracellular pH of either metabolic or respiratory origin. During the nine periods of study (five in the same patient) an attempt was made to change extracellular pH rapidly and observe the effect of this change on the serum potassium concentration. Extracellular pH was changed by appropriate therapy which included infusions of isotonic mixtures of NaCl, HCl, NH_4Cl, $NaHCO_3$ and sodium lactate. In one patient eight per cent CO_2 inhalation was used in combination with HCl infusion. In order to control the effect of changes in total body potassium on the serum potassium concentration experimental periods were short and an attempt was made to change potassium balance only slightly and in the direction opposite to the anticipated change in the serum potassium concentration. Because the caloric needs of none of the patients had been met during the periods antecedent to the experimental period, it was presumed that glycogen stores were depleted. During the experimental periods caloric needs were not met in order that potassium not be deposited with glycogen.

During the period of pH change sodium, potassium, chloride and nitrogen balances were determined in the usual manner. All patients were parenterally fed and none had stools during the period of study.

Femoral venous blood samples were considered to reflect more accurately than would arterial blood samples the pH of the extracellular space. Whole blood pH was determined anaerobically within ten minutes at 38° centigrade using a Cambridge pH meter with a glass electrode. Duplicate determinations, bracketed by standards, agreed within 0.02 pH unit. Sodium and potassium concentrations were determined by Baird internal standard flame photometry. Duplicate potassium determinations agreed within 0.1 mEq. per L. Chloride was determined by a modification of the Schales' mercury titration method (8). Plasma bicarbonate was determined by a modification of the Van Slyke titration (9). Serum and urine non-protein nitrogen was determined by the method of Koch and McMeekin (10).

Calculations

1. Change in the intracellular potassium content in excess of nitrogen,

$$\Delta K_I = bK' - \Delta K_E, \qquad (1)$$

where:

bK' = Potassium balance corrected for nitrogen.
ΔK_E = Change in the extracellular potassium content.

2. Potassium balance corrected for nitrogen,

$$bK' = bK - (3 \times bN'), \qquad (2)$$

where:

bK = Potassium balance
3 = Factor relating potassium balance to nitrogen balance.
$bN' = bN - (\Delta NPN \times W)$

where:

bN = Nitrogen balance.
ΔNPN = Change in serum non-protein nitrogen.
W = Total body water estimated as 60% of body weight.

[1] Supported by grants from Abbott Laboratories, the National Institutes of Health (Grant No. H-2185) and the Washington State Heart Association.

[2] Supported as a research associate by a grant from the Schweppe Foundation.

[3] The effect of alterations of extracellular pH on the relationship between extracellular and intracellular potassium is probably related to intracellular buffering and therefore ultimately dependent upon intracellular pH change. However, since the latter cannot be measured, the relationship must be studied in terms of extracellular pH.

3. Change in extracellular potassium content,

$$\Delta K_E = K_{E_2} - K_{E_1}, \qquad (3)$$

where:

$K_{E_1} = K_1 \times E_1$

$K_{E_2} = K_2 \times E_2$

where:

K_1 = Initial potassium concentration in plasma.

K_2 = Final potassium concentration in plasma.

E_1 = Initial Volume of extracellular space calculated as 20% of body weight.

E_2 = Final Volume of extracellular space determined from changes in the chloride space.

RESULTS

The effect of extracellular pH change on the serum potassium concentration is illustrated (Figure 1) by the course of events in patient H. M. This patient had at the outset respiratory alkalosis associated with hepatic coma. Extracellular pH was altered by a combination of CO_2 inhalation and HCl infusion. No potassium was administered during the period of study and the cumulative potassium balance in excess of nitrogen at the end of four days was minus 63 mEq. The reciprocal relationship between extracellular pH and the serum potassium concentration is apparent. Also when the pH was normal the serum potassium concentration was normal both before and after a loss of about 50 mEq. of potassium in excess of nitrogen. This observation is in agreement with the opinion that changes of less than 100 mEq. in potassium balance have a small effect *per se* on the serum potassium concentration (11).

The changes in serum electrolytes and electrolyte balances during the nine periods of study in five patients are presented in Table I and the important changes are summarized in Table II. In each instance a change in extracellular pH was associated with an inverse change in serum potassium concentration. Changes in intracellular potassium were small and in all but one instance opposite in direction to the change in serum potassium concentration. In the last column of Table II the change in serum potassium concentration per 0.1 unit change in extracellular pH has been calculated for each period of study. The range for four periods of correction of acidosis was 0.50 mEq. per L. to 1.20 mEq. per L. change per 0.1 pH change. The mean was 0.68 mEq. per L. change per 0.1 pH change. For correction of alkalosis the range was from 0.40 mEq. per L. to 1 mEq. per L. change pH 0.1 unit pH change. The

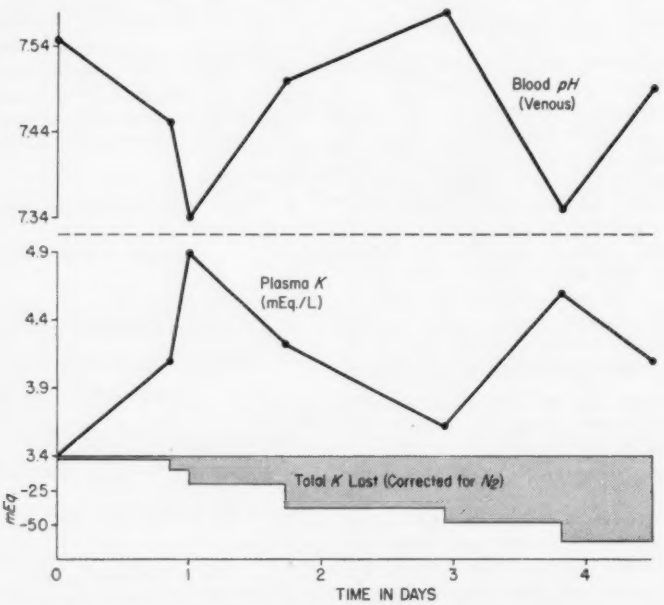


FIG. 1. SERIAL CHANGES IN BLOOD pH, PLASMA POTASSIUM CONCENTRATION AND POTASSIUM BALANCE IN PATIENT H. M.

TABLE I

Data of patients undergoing changes of extracellular pH *

Patient	Previous disorder	Body wt. Kg.	Duration of exp.	Type of therapy	Intake Na	K	Cl	HCO_3^-	N gm.	Output Na	K	Cl	N gm.	Balance bCl	bK	bN gm.	bK'†		NPN mg. %	Serum pH	K	Na	Cl	HCO_3
H. M. 1.	Resp. alk.	68	18 hr.	CO_2	0	0	0	0	0	44	12	42	4.3	− 42	−12	−4.3	− 4	Initial	23	7.55	3.4	142	116	19
																		Final	19	7.45	4.1	141	115	20
2.	Resp. alk.	—	18 hr.	HCl CO_2	0	0	208	0	0	28.5	27	54	2.8	+154	−27	−2.8	− 6	Initial	19	7.44	4.1	141	115	20
																		Final	29	7.34	5.1	137	118	13
3.	Metab. acid	—	14 hr.	—	0	0	0	0	0	—	28	43	5.5	− 43	−28	−5.5	−10	Initial	29	7.34	5.1	137	118	13
																		Final	31	7.50	4.2	135	117	16.4
4.	Resp. alk.	—	32 hr.	—	0	0	0	0	0	5	22	39	1.4	− 39	−22	−1.4	−18	Initial	31	7.50	4.2	135	117	16.4
																		Final	32	7.58	3.6	131	104	19.5
5.	Resp. alk.	—	17 hr.	HCl CO_2	0	0	160	0	0	1.6	13	20	0.8	+140	−13	−0.8	− 6	Initial	32	7.58	3.6	131	104	19.5
																		Final	36	7.35	4.6	126	104	14.9
J. R.	Metab. alk.	75	9.5 hr.	HCl NH_4Cl	0	0	360	0	2.5	29	21	52	2.6	+308	−21	−0.1	− 5	Initial	84	7.56	3.4	133	58	42.5
																		Final	96	7.43	3.9	129	79	29.2
H. P.	Metab. acid	70	19 hr.	Sodium bicarb.	650	40	40	650	0	9	3	3	—	+ 37	+37	−1.5	+32.5	Initial	—	7.02	6.8	148	104	2
																		Final	—	7.50	4.4	162	97	31
J. N.	Metab. acid	63	19 hr.	Sodium Lactate	300	0	0	0	0	0	0	0	0	0	0	0	0	Initial	103	7.20	9.3	132	106	11
																		Final	103	7.35	7.4	143	96	33
J. D.	Metab. acid	45	17 hr.	Sodium bicarb.	500	0	0	500	0	74	31	66	3.6	− 66	−31	−3.6	−15		BUN					
																		Initial	125	7.02	6.4	139	119	5
																		Final	131	7.49	4.0	146	116	17.5

* All electrolyte balance data are expressed in mEq. All serum electrolyte concentrations are expressed in mEq. per L.
† bK′ = Balance of potassium in excess of nitrogen.

TABLE II

Relationship between changes in extracellular pH and change in serum potassium concentration in nine experimental periods in five human subjects

Patient	Period	ΔK_s*	ΔpH	ΔK_I*	$\Delta K_s/0.1\Delta pH$
H. M.	1	+0.7	−0.10	−13	0.63
	2	+1.0	−0.10	−25	1.00
	3	−0.9	+0.16	+ 3	0.56
	4	−0.6	+0.08	−16	0.75
	5	+1.0	−0.23	−25	0.45
J. R.	1	+0.5	−0.13	−12.5	0.40
H. P.	1	−2.4	+0.48	+60	0.50
J. N.	1	−1.9	+0.15	+14	1.27
J. D.	1	−2.4	+0.47	+ 8	0.51

* ΔK_s = Change in the serum potassium concentration in mEq. per L.
ΔK_I = Change in intracellular potassium in excess of nitrogen.

mean was 0.58 mEq. per L. change per 0.1 pH change. The mean for both groups was 0.63 mEq. per L. change in serum potassium concentration per 0.1 unit change in extracellular pH.

DISCUSSION

There are several problems inherent in attempts to quantitate the relationship between extracellular pH change and change in the serum potassium concentration. First, metabolic as compared with respiratory pH alterations may involve different intracellular buffering mechanisms and thus be quantitatively different. In these studies no effort was made to separate metabolic and respiratory disorders of extracellular pH. Second, changes in the serum potassium concentration induced by changes in total body potassium or changes in glycogen stores introduce additional variables. Difficulty in control of these variables may account for the wide range of pH induced change in the serum potassium concentration reported here.

In these studies changes in intracellular potassium in excess of nitrogen were in the opposite direction as the change in serum potassium concentration. Thus, the change in serum potassium concentration attributed to change in extracellular pH in most instances might be slightly greater than that recorded.

The results demonstrate consistently that acidosis increases and alkalosis decreases the serum potassium concentration independently of changes in total body potassium. As discussed elsewhere (7), the human muscle analyses of Mudge and Vislocky (12) offer further support for the validity of this interrelationship.

Demonstration of this interrelationship between the serum potassium concentration and extracellular pH has several important clinical implications:

1) If the effect of pH is considered when interpreting the serum potassium concentration as an index of potassium need, this concentration becomes a more accurate guide to potassium therapy (11).
2) Extracellular pH must be controlled during experimental evaluation of other factors considered to influence the serum potassium concentration.
3) In the management of acute renal failure correction of acidosis and production of alkalosis will lower the serum potassium concentration and may facilitate prevention of potassium intoxication (cases H. P. and J. N.).

SUMMARY AND CONCLUSIONS

1. The effect of extracellular pH change on the relationship between the serum potassium concentration and intracellular potassium has been studied in 5 patients. A total of 9 experimental periods is described.

2. The results confirm the hypothesis that acidosis increases and alkalosis decreases the serum potassium concentration independently of changes in intracellular potassium. For every 0.1 unit change in extracellular pH there was an average inverse change of 0.6 mEq. per L. in the serum potassium concentration.

ACKNOWLEDGMENT

The authors are grateful to Miss Margaret Brown, Miss Marjorie Ferguson, Miss Patricia Hoover, and Miss Elma Lile for technical assistance.

REFERENCES

1. Fenn, W. O., and Cobb, D. M., The potassium equilibrium in muscle. J. Gen. Physiol., 1934, **17**, 629.
2. Keating, R. E., Weichselbaum, T. E., Alanis, M., Margraf, H. W., and Elman, R., The movement of potassium during experimental acidosis and alkalosis in the nephrectomized dog. Surg. Gynec. & Obst., 1953, **96**, 323.
3. Pitts, R. F., Mechanisms for stabilizing the alkaline reserves of the body. Harvey Lectures 1952–53, New York, Academic Press, 1954, p. 172.

4. Swan, R. C., and Pitts, R. F., Neutralization of infused acid by nephrectomized dogs. J. Clin. Invest., 1955, **34**, 205.
5. Giebisch, G., Berber, L., and Pitts, R. F., the extrarenal response to acute acid-base disturbances of respiratory origin. J. Clin. Invest., 1955, **34**, 231.
6. Stanbury, S. W., and Thomson, A. E., The renal response to respiratory alkalosis. Clin. Sc., 1952, **11**, 357.
7. Scribner, B. H., Fremont-Smith, K., and Burnell, J. M., The effect of acute respiratory acidosis on the internal equilibrium of potassium. J. Clin. Invest., 1955, **34**, 1276.
8. Scribner, B. H., Bedside determination of chloride: A method for plasma, urine and other fluids and its application to fluid balance problems. Proc. Staff Meet., Mayo Clin., 1950, **25**, 209.
9. Scribner, B. H., and Cailluoette, J. C., Improved method for the bed-side determination of bicarbonate in serum. J. A. M. A., 1954, **155**, 644.
10. Koch, F. C., and McMeekin, T. L., A new direct nesslerization micro-Kjeldahl method and a modification of the Nessler-Folin reagent for ammonia. J. Am. Chem. Soc., 1924, **46**, 2066.
11. Scribner, B. H., and Burnell, J. M., Interpretation of the serum potassium concentration. Metabolism, In press.
12. Mudge, G. H., and Vislocky, K., Electrolyte changes in human striated muscle in acidosis and alkalosis. J. Clin. Invest., 1949, **28**, 482.

SPECIAL NOTICE TO SUBSCRIBERS

Post Offices will no longer forward the Journal when you move.

Please notify The Journal of Clinical Investigation, Business Office, 622 West 168th Street, New York 32, N. Y. at once when you have a change of address, and do not omit the zone number if there is one.

PROLONGED HYPOTHERMIA IN EXPERIMENTAL PNEUMOCOCCAL PERITONITIS [1]

By B. EISEMAN, W. G. MALETTE, R. S. WOTKYNS, W. B. SUMMERS, AND J. L. TONG

(From the Department of Surgery, University of Colorado School of Medicine and Denver Veterans Administration Hospital, Denver, Colo.)

(Submitted for publication April 2, 1956; accepted May 10, 1956)

The recent use of generalized body hypothermia as an adjunct to anesthesia has prompted the application of this technique to the management of various types of infections. The isolated case reports on the use of hypothermia in clinical infections have attributed varying degrees of success to the use of this agent, but these studies in general have not been adequately controlled, nor has the mechanism of such benefit been properly investigated (1–9).

The rationale for the use of chronic hypothermia in the management of infectious processes rests upon the fact that the metabolism of bacteria pathogenic to man is reduced at temperature levels below that of normal body temperature. Reduction of the temperature of the animal might retard bacterial growth with possible benefit to the host. Such benefit would occur only if host resistance is depressed less than is bacterial growth.

The effect of cold on the resistance to infection was first noted in 1878 by Pasteur, Joubert, and Chamberland (10), who found that immersion of an unanesthetized fowl in cold water increased the susceptibility of the animal to subsequent anthrax infection. Thereafter numerous studies were carried out on the effect of body chilling (11) or the local application of cold (12) on host resistance to infection. Other studies have been reported on the *in vitro* alteration of phagocytosis by reduced temperatures (11, 13–18).

The purpose of this experiment is to determine the effect of prolonged (24-hour) generalized body hypothermia on the course of experimental peritonitis in anesthetized mice. It is emphasized that these animals were subjected to hypothermia after narcosis. This resulted in a metabolic situation very different from that obtained by the techniques used in other investigations (10–13) of chilling the unanesthetized animal. Another metabolic and immunologic difference of prime importance is that the animals in this study underwent prolonged generalized body hypothermia, not mere local cooling as used in some experiments (12).

MATERIALS AND METHODS

A total of 329, six to eight-week old (25 to 30-gram) Webster strain white mice [2] with type III pneumococcal peritonitis were included in this study. Of these, 160 animals were kept in a hypothermic state for 24 hours, while 130 animals served as simultaneous normothermic controls. Thirty-nine animals with experimental peritonitis received anesthesia according to the same schedule as the hypothermic group but were not cooled. Experimental runs were made on groups of 20 animals at a time, half being selected at random as controls.

The organisms [3] were maintained at a standard virulence by weekly mouse passage. The inoculum was prepared by plating on blood agar for 24 hours at 37° C. This plate was placed in a refrigerator (4° C.) until ready for sub-culture. In no instance did this period exceed 6 days. Prior to use 1 to 3 of the smoother mucoid colonies were inoculated into 15 to 20 ml. of trypticase soy broth in a *25* by *150*-mm. test tube and incubated at 37° C. for 18 hours. At the end of this period there was obvious diffuse turbidity. The inoculum was drawn into a 1-ml. dry tuberculin syringe and 0.5 ml. injected intraperitoneally through a No. 26 hypodermic needle into each animal.

The *in vitro* growth of pneumococci at varying temperatures was measured by determining the turbidity of a broth culture at hourly intervals on a Beckman B. spectrophotometer at 470 λ. A preliminary absorption curve was plotted on uninoculated broth. Growth was plotted as the reciprocal of turbidity. Cultures were maintained in a constant temperature incubator set either at 37.5° or 19° C.

After all of the animals had been inoculated they were arbitrarily divided into a control and hypothermic group—

[1] This work supported in part by a grant from the American Heart Association to the Department of Surgery, University of Colorado School of Medicine.

[2] Obtained from Colorado Serum Company, Denver, Colorado.

[3] *D. pneumoniae III* originally obtained from the National Institutes of Health.

thus producing simultaneous paired experiments in each run. Animals in each group were identified by alcoholic-picric acid solution skin markings.

The control animals were placed in separate jars containing adequate food and water at room temperature and observed at 6-hour intervals.

The hypothermic animals were placed in similar jars for 2 hours following inoculation, following which each animal received a subcutaneous abdominal wall injection of 0.25 to 0.30 ml. of 1.0 per cent saline solution of sodium pentobarbital (Nembutal®, Abbott). This inoculum provided 0.1 mg. of pentobarbital per gram of body weight.

After 30 minutes the animals were unconscious and were placed on a metal pan in the cold room at 4° C. for 6 to 8 minutes. The hypothermic animals were then immediately placed in a constant temperature box [4] adjusted to 19.5° C.

Mouse body temperatures were recorded by means of plastic covered No. 24 gauge constantin wire thermocouples previously greased with petroleum jelly and inserted 2 to 3.5 cm. into the rectum of each animal. Each electrode led into a constant recording Brown potentiometer (Model Y 153 × 60 PA a-x-iv) via an electric-clock-operated selector switch which serially recorded the temperature of each animal and of the constant temperature box at 10-minute intervals.

Rectal temperatures reached a constant level of 21° to 24° C. within 30 minutes after the animals were placed in the constant temperature box. When an animal began to emerge from anesthesia its body temperature was noted to rise gradually.

Subsequent additional anesthesia was administered according to the following dosage schedule, as required to maintain narcosis: 0.05 ml. of a 1 per cent pentobarbital saline solution was given subcutaneously into the back, at not less than 20-minute intervals. Two to six such inoculations were variously required over the first 3 hours of hypothermia in order to obtain adequate narcosis. Individual variation was wide and did not correlate with body weight.

[4] Manufactured by Precision Scientific Co., Chicago Ill., No. 9040.

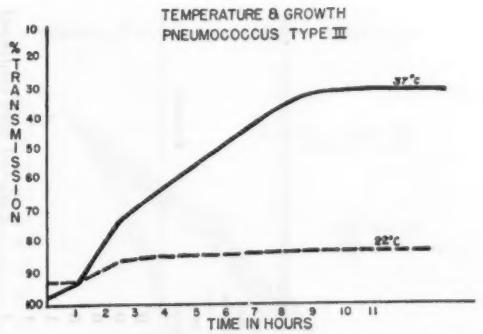


FIG. 1. EFFECT OF HYPOTHERMIA ON *in vitro* GROWTH OF PNEUMOCOCCI III

If, at the end of 3 to 5 hours following the original anesthesia, the animal appeared to be emerging from anesthesia (response to pinching of tail or ear), 0.20 to 0.25 ml. of a 1 to 3 per cent saline phenobarbital solution was given subcutaneously in the back. This combination of anesthesias in the presence of hypothermia served to maintain narcosis for 24 hours.

Twenty-four hours after the induction of hypothermia the animals were removed from the constant temperature box and placed in glass jars at room temperature and allowed to warm spontaneously. In 1 to 4 hours the animals awoke, were running about their cages, and body temperatures returned to normal (37.2° C.).

In each instance where a control animal died during the first 24 hours following inoculation or where a hypothermic animal died within 48 hours following inocu-

TABLE I

Survival following experimental peritonitis—All deaths included *

	12 Hours		24 Hours		36 Hours		48 Hours		60 Hours		72 Hours		84 Hours		96 Hours	
Experiment number	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C
15 (10 H & 10 C)	9	10	5	9	4	4	3	0	3	0	0	0	0	0	0	0
16 (9 H & 10 C)	9	10	7	10	6	2	4	2	2	0	0	0	0	0	0	0
17 (20 H & 20 C)	15	20	14	13	14	0	13	0	12	0	9	0	7	0	0	0
18 (15 H & 20 C)	12	20	10	18	10	13	10	1	10	0	8	0	5	0	2	0
19 (10 H & 10 C)	10	10	8	10	8	9	7	1	7	0	5	0	2	0	0	0
20 (20 H & 20 C)	20	20	18	16	12	11	11	0	11	0	9	0	4	0	0	0
21 (20 H & 10 C)	20	10	13	10	12	6	9	2	8	2	1	2	0	2	0	2
22 (20 H & 10 C)	20	10	7	10	7	10	6	1	6	1	6	0	3	0	0	0
23 (20 H & 10 C)	20	10	11	5	9	4	5	0	3	0	1	0	0	0	0	0
24 (20 H & 10 C)	20	10	11	9	10	6	9	0	6	0	0	0	0	0	0	0
Total No. Living	155	130	104	110	92	65	77	7	68	3	39	2	21	2	2	2
Total No. Dead	9	0	60	20	72	65	87	123	96	127	125	128	143	128	162	128

* H = Hypothermia.
C = Control.

TABLE II

Survival following experimental peritonitis—Anesthesia and accidental deaths excluded *

Experiment number	12 Hours H	12 Hours C	24 Hours H	24 Hours C	36 Hours H	36 Hours C	48 Hours H	48 Hours C	60 Hours H	60 Hours C	72 Hours H	72 Hours C	84 Hours H	84 Hours C	96 Hours H	96 Hours C	Hypothermia anesthesia and accidental deaths 12 Hours	Hypothermia anesthesia and accidental deaths 24 Hours	Remarks
15 (9 H & 10 C)	9	10	5	9	4	4	3	0	3	0	0	0	0	0	0	0	1	0	
16 (9 H & 10 C)	9	10	7	10	6	2	4	2	2	0	0	0	0	0	0	0	0	0	
17 (15 H & 20 C)	15	20	14	13	14	0	13	0	12	0	9	0	7	0	0	0	5	0	
18 (12 H & 20 C)	12	20	10	18	10	13	10	1	10	0	8	0	5	0	2	0	3	0	
19 (9 H & 10 C)	9	10	8	10	8	9	7	1	7	0	5	0	2	0	0	0	0	1	
20 (19 H & 20 C)	19	20	18	16	12	11	11	0	11	0	9	0	4	0	0	0	0	1	
21 (16 H & 10 C)	16	10	13	10	12	6	9	2	8	2	1	2	0	2	0	2	0	4	2 control mice continued to live
22 (7 H & 10 C)	7	10	7	10	7	10	6	1	6	1	6	0	3	0	0	0	0	13	13 hypothermic mice accidentally drowned
23 (13 H & 10 C)	13	10	11	5	9	4	5	0	3	0	1	0	0	0	0	0	0	7	California Webster strain of mice used
24 (14 H & 10 C)	14	10	11	9	10	6	9	0	6	0	0	0	0	0	0	0	0	6	California Webster strain of mice used
																	Total 9	32	Grand Total of 41 deaths due to technical difficulties
Total No. Dead	0	0	19	20	31	65	46	123	55	127	84	128	102	128	121	128			
Total No. Living	123	130	104	110	92	65	77	7	68	3	39	2	21	2	2	2			
Std. Deviation	0	0	4.23	3.74	3.1	4.17	3.49	.82	3.49	.67	3.90	.63	2.55	.63	.63	.63			
x^2			1.89×10^{-4}		16.5		93.27		85.24		40.15		19.89		0.203				
P		†	.99		<.001		<.001		<.001		<.001		<.001		.50–.70				

* H = Hypothermia C = Control.

† x^2 need be only 10.82 for P = .001.

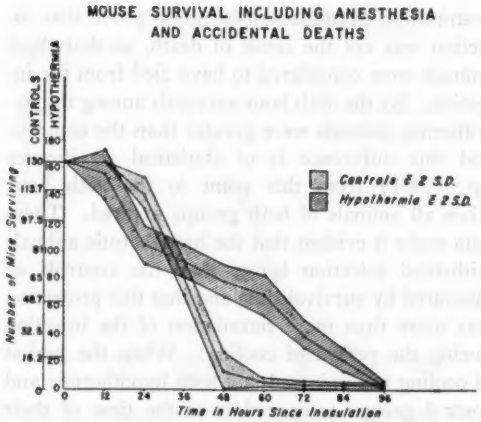


FIG. 2. SURVIVAL FOLLOWING EXPERIMENTAL PERITONITIS—ALL DEATHS INCLUDED

Shaded area represents 2 S.D. on each side of mean.

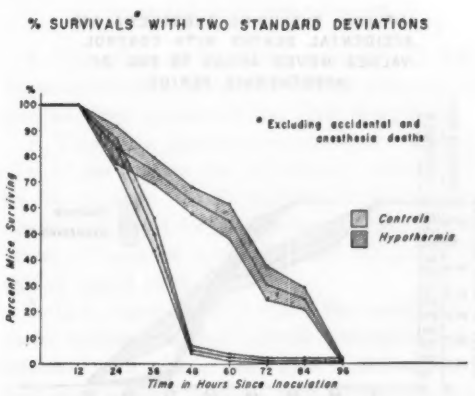


FIG. 3. PERCENTAGE SURVIVALS—EXCLUDING ANESTHESIA AND ACCIDENTAL DEATHS

Shaded area represents 2 S.D. on each side of mean.

lation an autopsy was performed and the cause of death ascertained. This obviated misinterpretation of deaths due to peritonitis from those due to accidental causes or from overdose of anesthesia.

RESULTS

The comparison of pneumococcal growth at 37.5° C. and at 21° C. over a 24-hour period is recorded in Figure 1. It is evident that hypothermia markedly diminishes the rate of bacterial growth.

The dose of pneumococci chosen for this series of experiments purposely was adjusted to cause death in the test animals within a 4-day period. Long-term survivals therefore did not occur. The number of survivors in both the control and hypothermic group is indicated in Table I and Figure 2, which includes all animals included in the study. The statistical analysis of even these raw data indicates hypothermia delays the progress of the course of the infection.

Table II and Figure 3 record the survival rates of the animals, excluding the 41 animals who died from the anesthesia or by some technical accident. Thirteen of these deaths were due to accidental drowning in the constant temperature box (Experiment No. 22). In each case included in this group of 41 animals, postmortem examination confirmed that the animals did not die from pneumococcal peritonitis. These data are shown in Table II. Statistical analysis of these data shows an even more prominent survival of the hypothermic animals, since the accidental and anesthetic deaths

TABLE III

Survival following experimental peritonitis—Excluding anesthesia and accidental deaths with control values moved ahead to end of period of hypothermia

Time (*hrs.*)		Hypothermia		Controls			
Hypothermia	Controls	Survival	Standard deviation	Survival	Standard deviation	χ^2	P
0		123	0				
12		123	0				
24	0	104	4.23	130	0	5.67	<0.02
36	12	92	3.10	130	0	16.27	< .001
48	24	77	3.49	110	3.74	19.48	< .001
60	36	68	3.49	65	4.17	0.704	<0.5
72	48	39	3.90	7	0.82	19.05	< .001
84	60	21	2.55	3	0.67	5.07	>0.02
96	72	2	0.63	2	0.63	.0195	0.9

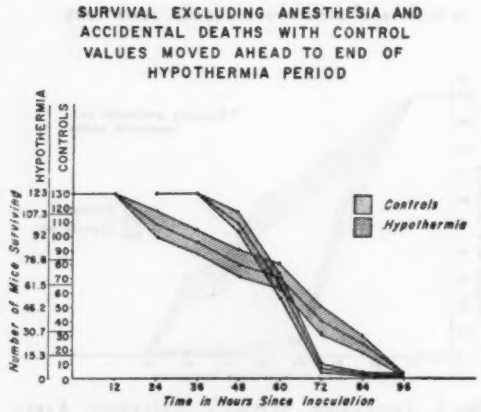


FIG. 4. PERCENTAGE SURVIVALS WITH CONTROL MICE MOVED AHEAD TO END OF PERIOD OF HYPOTHERMIA (24 HOURS)

Shaded area represents 2 S.D. each side of mean.

were all from the hypothermic group. It is evident that the hypothermic animals survive longer than the controls and that this survival is statistically significant.

The period of hypothermia was 24 hours and the foregoing data indicate that hypothermia retarded the progress of infection. In order to determine whether hypothermia did more than merely retard the infection during the period of cooling, animal survivals were plotted with the control animal data moved ahead 24 hours so that determination of survival would begin simultaneously with the hypothermic animals as the latter were removed from their cool environment and began to warm spontaneously (Table III and Figure 4). Such a maneuver weighs survival in favor of the controls, since 19 of the 123 (15 per cent) of the hypothermic animals had expired during anesthesia. None of these animals died because of an obvious accident and in none could postmortem examination definitely prove that infection was not the cause of death, so that these animals were considered to have died from the infection. By the 48th hour survivals among the hypothermic animals were greater than the controls, and this difference is of statistical significance ($p < 0.001$) from this point to the 96th hour when all animals of both groups expired. These data make it evident that the hypothermic animals withstood infection better than the controls as measured by survival time and that this protection was more than mere retardation of the infection during the period of cooling. When the period of cooling is eliminated and both hypothermic and control groups compared from the time of their normothermic conditions, the animals previously cooled outlive their controls.

The control animals given a course of anesthesia similar to the hypothermic animals, but not cooled, expired at a rate similar to the unanesthetized normothermic controls (Table IV and Figure 5). This indicates that the anesthesia did not affect the course of the infection. It was necessary to maintain the anesthetized mice in a carefully warmed room, for it was found that the animals' body temperature dropped to hypothermic levels on relatively short exposure to a cool environment.

DISCUSSION

According to these data hypothermia is associated with prolongation of survival following experimental pneumococcal peritonitis. Cooling depresses *in vitro* bacterial growth and it is to be expected that a similar effect occurs in the infected hypothermic animal. Of importance, as far as ultimate clinical value however, is the relative effect of hypothermia on bacterial growth compared to any change in host immunologic response. Should hypothermia hold back bacterial growth

TABLE IV

Infected warm anesthetized mice compared with infected controls

Time in hours	0 Hours		12 Hours		24 Hours		36 Hours		48 Hours		60 Hours	
	C	A	C	A	C	A	C	A	C	A	C	A
Number Living	130	39	130	39	116	32	65	21	3	1	2	0
Number Dead	0	0	0	0	20	7	65	18	127	38	128	39
% Living	100	100	100	100	80.8	82.0	50.0	53.8	5.4	2.6	2.3	00.0
% Dead	00.0	00.0	00.0	00.0	19.2	18.0	50.0	46.2	94.5	97.4	97.7	100.0

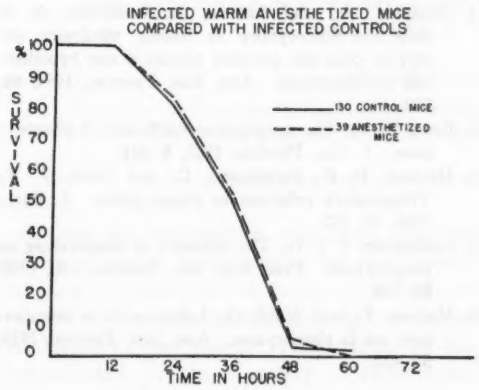


FIG. 5. SURVIVAL OF NORMOTHERMIC CONTROLS COMPARED TO NORMOTHERMIC ANIMALS GIVEN ANESTHESIA IN A SCHEDULE SIMILAR TO THE HYPOTHERMIC GROUP

Shaded area represents 2 S.D. on each side of mean.

and immunologic response at the same rate, no host benefit will be attained in the subject not otherwise treated with bactericidal substances. Thus, if unaltered bacterial growth is resumed with the return of normothermia, and bacterial defense mechanisms have been depressed to a similar degree during the period of cooling, no increased host survival can be anticipated.

Animal survival following infection is determined both by the characteristics of bacterial growth and the immunologic reaction of the host. Since the hypothermic animals in this study had a longer survival than could be accounted for merely by depression of bacterial growth during the period of cooling, it can be concluded that diminution in host resistance is less than reduction in the rate of bacterial growth.

In this study we were unable to determine the absolute nature of the degree of alteration in the immunologic response of the hypothermic animal, since only the combined effect of infection and host resistance has been measured. Studies are currently in progress in which the effect of hypothermia on the host resistance factors, such as the phagocytic index, are being analyzed.

By experimental design no long-term animal survivals have resulted from these studies, but length of survival appears to be a valid relative measurement of bacterial and host response to hypothermia.

SUMMARY AND CONCLUSIONS

1. The effect of hypothermia at 19° C. for a 24-hour period on anesthetized mice with type III pneumococcal peritonitis has been described.

2. There is a statistically significant longer period of survival in the hypothermic animal than in the controls. Such survival is greater than can be accounted for merely on the basis of inhibition of bacterial growth during the actual period of hypothermia.

3. It is suggested that under the conditions of these experiments that bacterial multiplication is inhibited at a rate greater than is diminution of host immunologic response with subsequent beneficial effects on the length of host survival.

ACKNOWLEDGMENT

Our thanks are due to Mr. D. Stouder, C. Hlad, and F. G. Painter for their technical assistance in this investigation.

REFERENCES

1. Balch, H. H., Noyes, H. E., and Hughes, C. W., The influence of hypothermia on experimental peritonitis. Surgery, 1955, **38**, 1036.
2. Boudin, G., Barbizet, J., Herman, J., and Binet, J.-L., Réflexions sur un cas de tétanos guéri, Traite successivement par hibernation artificielle et par curare de synthèse. Bull. et mém. Soc. méd. d. hôp. de Paris, 1953, **69**, 31.
3. Chesni, Y., Hibernothérapie et grands dérèglements neurovégètatifs nonchirurgicaux. Réflexions sur 17 cas de tétanos traités par l'hibernation. Revue neurol. 1953, **89**, 491.
4. Contzen, H., Die Behandlung des manifesten tetanus im künstlichen Winterschlaf. Arch. klin. chir., 1954, **278**, 27.
5. Gomez, F., and Gelin, G., Bronchopneumonie coquelucheuse hyperthermique traitée avec succès par l'hibernation. Bull. et mém. Soc. med. d. hôp. de Paris, 1953, **69**, 481.
6. Höring, F. O., Beiträge zur Theorie der Schlaf- und Winterschlaf-therapie von Infektionskrankheiten. Deutsche med. Wchnschr., 1954, **79**, 987.
7. Lavergne, V. De, and Schmitt, J., Scarlatine puerpérale maligne: hibernation, guérison. Bull. et mém. Soc. med. d. hôp. de Paris, 1953, **69**, 520.
8. Martin, C., Sur l'hibernation artificielle appliquée au traitement d'un cas très sévère de septico-pyohémie à staphylocoques. Presse méd., 1953, **61**, 84.
9. Mollaret, P., Bastin, R., Pocidalo, J. J., and Besançon, F., Premier essai d'hibernation dans le tetanos

gravissime. Premier échec. Bull. et mém. Soc. méd. d. hôp. de Paris, 1952, **68**, 381.

10. Pasteur, L., Joubert, J. F., and Chamberland, C., La théorie des germes et ses applications à la médicine et à la chirurgie. Bull. Acad. de méd., Paris, 2d Ser., 1878, **7**, 432.
11. Rolly, F., and Meltzer, Experimentelle untersuchungen uber die Bedeutung der Hyperthermi. Deut. Arch. Klin. Med., 1908, **94**, 335.
12. Bruneau, J., and Heinbecker, P., Effects of cooling on experimentally infected tissues. Ann. Surg., 1944, **120**, 716.
13. Cottingham, E., and Mills, C. A., Influence of environmental temperature and vitamin-deficiency upon phagocytic functions. J. Immunol., 1945, **47**, 493.
14. Delaunay, A., and Lebrun, J., Inhibition de la diapédèse leucocytaire et lésions viscérales observées chez des animaux soumis a une hypothermie expérimentale. Ann. Inst. Pasteur., 1954, **86**, 520.
15. Fenn, W. O., The temperature coefficient of phagocytosis. J. Gen. Physiol., 1921, **4**, 331.
16. Harmon, D. R., Zarafonetis, C., and Clark, P. F., Temperature relations in phagocytosis. J. Bact., 1946, **52**, 337.
17. Ledingham, J. C. G., The influence of temperature on phagocytosis. Proc. Roy. Soc., London, s.B., 1908, **80**, 188.
18. Madsen, T., and Wulff, O., Influence de la température sur la phagocytose. Ann. Inst. Pasteur, 1919, **33**, 437.

THE URINARY EXCRETION OF SODIUM AND POTASSIUM DURING THE PYROGENIC REACTION IN MAN [1, 2]

By WILLOUGHBY LATHEM [3]

(From the Veterans Administration Hospital, West Haven, Connecticut, and The Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.)

(Submitted for publication December 7, 1955; accepted May 10, 1956)

Among the mechanisms regulating the renal tubular transport of electrolytes the renal circulation plays an important but as yet imperfectly understood role. An interrelation between renal hemodynamics and sodium reabsorption has been demonstrated (2) in a variety of experimental circumstances associated with renal vasoconstriction (infusions of adrenal medullary hormones [3], neosynephrine [4], and other drugs [5]; during orthostasis [4], hemorrhage [6]). Whether such an interrelation exists during renal hyperemia is, however, not known. The present study was undertaken in order to examine this problem further. For this purpose a study was made of urinary sodium and potassium excretion during the pyrogenic reaction, a physiologic alteration accompanied by both renal vasoconstriction and vasodilatation (7).

METHOD

Thirty-two studies were made in 27 convalescent adult male subjects. All subjects were free from cardiovascular or renal disease, none was recovering from a febrile disorder, and all were maintained on a normal hospital diet containing approximately 10 grams of sodium chloride a day. All studies were made in fasting, recumbent subjects during the morning hours, from approximately 8 A.M. until noon, or later as noted.

The effects of the pyrogenic reaction were examined in 16 subjects. In eleven of these studies the clearances of inulin, PAH, sodium and potassium were determined before and following administration of typhoid-paratyphoid vaccine. All 11 subjects were given 500 ml. of water by mouth one-half to one hour prior to the test and received an infusion of 5 per cent dextrose in water intravenously at a rate of 5 ml. per minute throughout the test. Urine was collected every 10 to 30 minutes over a period of approximately 3 hours. Typhoid vaccine (75,000,000 organisms) was administered intravenously at the conclusion of the first 10 to 20-minute collection period. Blood was collected without stasis at approximately half-hour intervals throughout the test. In order to prevent a rise in body temperature 8 of these subjects were given Aminopyrine on the day prior to the test in a dosage of 0.6 gram every four hours. Three subjects (J. P., E. V. and G. R.) did not receive this medication.

In five subjects the effects of the pyrogenic reaction on sodium and potassium excretion were observed over a five-hour period and measurements of renal hemodynamics were not made. These subjects ingested 500 ml. of water one-half to one hour prior to the test and approximately 100 to 200 ml. every hour thereafter in order to maintain an adequate urine flow. No intravenous fluids were given. Urine was collected every hour by spontaneous voiding which usually required that the subject stand briefly by the side of the bed. Typhoid-paratyphoid vaccine (75,000,000 organisms) was administered intravenously at the end of the first hourly collection period. All five subjects were premedicated with Aminopyrine.

Control observations of urinary sodium and potassium excretion were made in 16 studies under conditions similar to those employed in the experimental (pyrogen) studies except that typhoid vaccine was not administered. None of these subjects received Aminopyrine.

In all studies the body temperature was measured orally and the cardiac rate and arterial blood pressure (sphygmomanometer) were determined at frequent intervals. Sodium and potassium content of urine and plasma were determined by an internal standard flame photometer. Inulin was determined by the method of Harrison (8) and PAH by the method of Smith, Finkelstein, Aliminosa, Crawford, and Graber (9).

RESULTS

Body temperature and subjective response

In eleven of thirteen subjects premedicated with Aminopyrine the body temperature remained normal following the injection of typhoid vaccine (Tables I and II). These subjects remained asymptomatic throughout the test. In two (J. McN. and R. B.) premedicated and in three

[1] A preliminary report of this study has appeared in abstract form (1).

[2] Supported by grants from The American Heart Association and the Greenwich Health Association and the National Heart Institute, National Institutes of Health, Public Health Service.

[3] Present address: Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

(J. P., E. V. and G. R.) untreated subjects a chill occurred approximately one to two hours after the administration of vaccine and the oral temperature increased to approximately 100° F. (Table I). These subjects complained of headache, backache and malaise at the time of the chill.

The arterial blood pressure increased 5–10/5–10 mm. Hg in most subjects, along with an acceleration of cardiac rate (10 to 20 beats per minute), during the initial phase of the pyrogenic reaction (one to two hours) and remained elevated throughout the study (Table I).

Renal hemodynamics

The renal circulatory adjustments accompanying the pyrogenic reaction were similar to those reported in previous studies (10, 11). Renal plasma flow (PAH clearance) increased (on the average, 40 per cent) one and one-half to two hours following administration of typhoid vaccine in all eleven subjects in whom renal hemodynamics were studied (Table I). Glomerular filtration rate (inulin clearance) did not change from resting values during hyperemia (except in A. R. in whom there was a slight decrement and in E. V. in whom

TABLE I

Renal function and urinary electrolyte excretion in eleven normal subjects during the pyrogenic reaction *

Subject Age	S.A.	Elapsed time *min.*	Temp. °F.	B.P. *mm. Hg*	Plasma N_a *mEq./L.*	Plasma K *mEq./L.*	C_{in} *ml./min.*	C_{pah} *ml./min.*	FF %	V *ml./min.*	$U_{Na}V$ *μEq./min.*	U_kV *μEq./min.*
W. G. W.		46	97[4]	104/62	137.8	4.42	115	545	21	4.3	63.4	67.0
45	1.83	79					105	468	22	1.5	59.2	56.8
		104					108	507	21	2.0	22.4	39.3
		127			138.1	3.83	110	572	19	3.5	50.4	31.9
		147					111	769	14	2.1	80.2	34.5
		170	97[8]	106/50			108	813	13	3.2	54.8	51.1
J. Da.		44	97[2]	110/80	136.0	4.12	150	735	20	7.1	142.8	101.0
32	2.16	75					134	664	20	4.2	114.2	80.0
		102	97[4]	112/90	136.4	4.20	134	599	22	0.6	71.6	57.8
		127		134/90			154	752	20	0.6	108.2	42.4
		151			134.5	3.65	161	1,042	15	2.5	191.4	45.4
		169					162	1,173	14	5.8	158.5	68.8
		188	97[6]	146/90	136.8	3.60	149	1,052	14	8.9	134.8	87.0
W. R. W.		48	97[2]	110/72	137.4	4.30	132	723	18	11.2	96.7	96.9
33	1.91	82	97[0]	110/80			105	540	19	3.8	77.7	62.0
		99					119	776	15	0.8	57.2	61.4
		114					111	1,059	11	0.9	100.6	95.2
		131			139.6	4.20	134	1,256	11	1.4	81.2	56.9
		143					135	1,225	11	1.2	102.3	58.0
		158	98[6]	120/80	136.5	4.05	140	1,232	11	1.5	113.9	61.7
S. P.		54	97[0]	90/58	135.5	4.06	161	752	21	0.8	29.0	99.5
24	1.95	86		90/58			153	668	23	2.1	15.0	89.0
		109			137.2	3.80	170	777	22	2.0	12.1	77.6
		154	97[2]				168	903	18	6.8	65.0	60.1
		168					161	1,041	15	9.7	137.5	43.8
		181	97[2]	120/70	136.5	3.63	168	1,206	14	9.8	161.5	46.9
J. McN.		72	98[0]	102/50	133.4	4.25	108	756	14	0.6	11.1	73.0
37	1.64	102	100[2]	106/58	130.0	3.92	159	1,101	14	0.7	22.2	101.5
		123					116	1,080	15	0.9	10.2	70.9
		138		108/58	131.2	3.70	110	1,036	11	1.3	6.2	70.9
		152					108	1,055	10	1.8	6.4	79.9
		168					96	968	10	2.3	5.5	76.4
		183	100[4]	120/60	132.0	3.45	103	1,016	10	3.1	5.5	82.1

* In each subject the initial values for renal hemodynamics and electrolyte excretion are the averages of three periods. All other values represent single determinations. The elapsed times are in reference to the beginning of the first collection period. Typhoid vaccine was administered intravenously at the end of the first collection period (10 to 20 minutes after the beginning of the test). All subjects except J. P., E. V., and G. R. were premedicated with Aminopyrine. Abbreviations are as follows: S.A. = body surface area (M^2), Temp. = body temperature (oral), B.P. = arterial blood pressure, C_{in} = inulin clearance—glomerular filtration rate (ml./min.), C_{pah} = p-aminohippurate clearance—renal plasma flow (ml./min.), FF = filtration fraction—C_{in}/C_{pah} (%), V = urine flow (ml./min.), $U_{Na}V$ = urinary sodium output (μEq./min.), U_kV = urinary potassium output (μEq./min.).

TABLE I—*Continued*

Subject		Elapsed time	Temp.	B.P.	Plasma Na	Plasma K	C_{in}	C_{pah}	FF	V	$U_{Na}V$	$U_{K}V$
Age	S.A.	*min.*	*° F.*	*mm. Hg*	*mEq./L.*	*mEq./L.*	*ml./min.*	*ml./min.*	%	*ml./min.*	*μEq./min.*	*μEq./min.*
	G. P.	56	97[6]	118/74	132.5	4.45	143	629	23	6.1	26.0	62.1
27	2.01	84		132/88			133	696	19	4.8	11.9	49.7
		116	98[4]		135.7	3.90	132	788	17	8.2	27.9	44.4
		138					126	856	15	10.2	40.0	39.6
		152					135	816	17	9.6	35.6	44.5
		168	98[6]	130/50	135.9	3.95	125	734	17	7.8	24.5	49.6
	R. B.	45	97[4]	128/78	135.5	3.85	161	797	20	10.9	45.3	52.1
26	1.90	71					133	640	21	4.7	44.9	28.0
		92	99[2]	140/82			171	822	21	0.6	45.6	8.6
		118			135.3	3.68	199	998	20	0.9	79.4	11.5
		141					180	1,061	17	4.0	70.3	55.2
		159					178	1,294	14	2.7	57.0	62.0
		179	99[8]	140/72	136.4	3.65	158	1,279	12	10.6	45.3	72.5
	A. R.	26	98[0]	130/90	137.1	3.98	112	549	20	10.8	118.8	75.4
30	1.82	43					84	434	20	8.3	95.0	69.1
		73					97	475	19	4.2	63.8	50.8
		95			138.6	3.78	98	513	19	2.2	30.5	39.0
		117					101	525	19	5.4	55.6	27.3
		143	98[0]	140/90			96	708	14	7.4	106.5	24.1
		164					96	817	12	6.9	158.0	31.7
		183	98[0]	140/90	139.2	3.70	90	740	12	2.5	181.0	46.5
	J. P.	35	97[4]	108/68	136.4	3.97	151	813	19	6.0	113.8	44.5
33	2.24	59					141	819	17	9.7	143.5	46.6
		83					129	700	18	3.4	140.0	36.6
		113	98[2]	118/74	137.8	3.80	140	741	19	1.2	141.6	32.8
		144					145	1,004	14	2.8	325.0	34.5
		162					131	1,146	11	3.5	384.0	48.6
		183	99[2]	120/80	137.4	3.70	115	1,122	10	2.3	208.0	70.8
	E. V.	36	98[0]	118/72	137.2	4.21	137	640	21	7.5	220.2	109.1
31	1.87	66					122	623	20	9.2	271.2	91.6
		98					128	608	19	3.5	289.0	78.4
		123	99[2]	120/60	139.0	4.10	119	618	19	1.4	220.0	69.6
		140					119	656	18	1.4	204.6	63.0
		157					140	812	17	2.2	287.5	65.8
		172	100[4]		138.3	3.85	149	909	15	2.3	317.0	70.8
	G. R.	34	97[8]	124/74	136.6	3.98	118	645	18	12.1	215.2	141.8
32	2.01	66					62	648	10	7.6	217.2	133.5
		97	99[4]	120/64	136.3	3.90	100	595	17	1.2	104.1	107.0
		125					117	534	22	1.1	116.0	80.6
		141					156	1,018	15	1.2	144.3	89.9
		159	100[2]	130/70			112	840	13	0.7	41.6	85.8
		175			136.5	3.90	118	967	12	1.1	71.0	113.0

it increased). In nine subjects the hyperemic phase was preceded by a transient reduction in renal plasma flow which occurred one to two hours following injection of the vaccine (average change, 13 per cent). In seven of these subjects a transient decrement (11 to 21 per cent; average change, 12 per cent) in glomerular filtration rate also occurred at this time; in the remaining two (W. G. W. and S. P.) the filtration rate did not change. The FF tended to remain the same or to increase slightly at this time. In two subjects (J. McN. and G. P.) neither glomerular filtration nor renal plasma flow changed during the latent period prior to hyperemia.

Urine flow

Urine flow decreased one to two hours following administration of the vaccine in eleven of sixteen subjects (V, Tables I and II). In the remainder (S. P., J. McN., G. P., W. L. and F. W.) urine volume was sustained or increased at this time. The reduction in urine flow was always accompanied by a fall in glomerular filtration rate in those studies in which renal hemodynamics were measured (Table I). The decrement in urine flow exceeded the change in filtration in all. In three studies (S. P., J. McN., and G. P.) glomerular filtration rate did not change prior to hyperemia and

urine volume increased or remained constant. During the later or hyperemic phase of the pyrogenic reaction (2 to 5 hours) no systematic change in urine volume was observed, although flow returned towards resting levels if previously depressed. At this time glomerular filtration returned to initial levels. No difference was noted between afebrile and febrile subjects during either the vasoconstrictive or hyperemic phases.

Sodium excretion

The changes in urinary sodium output were variable. During the initial phase of the pyrogenic reaction (one to two hours following administration of the vaccine) sodium excretion diminished transiently (32 to 93 per cent) in 9 of 16 subjects ($U_{Na}V$, Tables I and II). In other studies sodium output increased (four) or remained unchanged (three) at this time. These alterations were not significant when compared to control subjects and could not be correlated with changes in renal hemodynamics. Although a reduction in glomerular filtration rate accompanied the decrement in sodium output in four of seven studies (J. Da., W. R. W., A. R., and G. R.), sodium excretion increased in two subjects (E. V. and J. P.) and remained unchanged in one (R. B.) despite a fall in glomerular filtration rate. Moreover, in W. G. W., G. P. and S. P. sodium output fell 72, 54, and 48 per cent, respectively, without a significant change in glomerular filtration. There was no correlation between the changes in sodium excretion and renal plasma flow.

During the second to fifth hour following ad-

TABLE II

Urinary electrolyte excretion in five normal subjects during the pyrogenic reaction *

Subject		Elapsed time	Temp.	Plasma Na	Plasma K	V	$U_{Na}V$	U_KV
Age	S.A.	*min.*	*° F.*	*mEq./L.*	*mEq./L.*	*ml./min.*	*μEq./min.*	*μEq./min.*
W. L.		56	97.6	137.8	4.50	0.7	33.0	27.0
18	1.92	118				0.7	40.6	40.8
		181				0.6	37.3	45.9
		237	98.6			1.0	81.7	73.7
		299				2.3	85.6	143.9
		361				1.5	91.2	110.9
		417	98.6	137.6	4.50	1.5	94.6	93.7
F. W.		60	97.8	129.5	3.95	0.7	28.1	59.1
32	1.83	124				1.1	66.7	80.8
		183				2.0	40.1	52.0
		241				3.2	50.2	76.6
		316				2.0	62.8	112.4
		371				1.3	91.1	88.3
		423	98.0	139.5	3.87	3.6	56.9	65.8
H. H.		65	97.4	137.6	4.15	3.2	143.6	107.6
46	1.86	121				4.8	212.0	132.1
		182				2.0	97.4	78.6
		262				4.1	236.5	65.0
		303				4.6	153.6	131.4
		366	98.2	131.8	3.75	5.1	107.3	249.0
D. B.		66	97.4			8.8	68.4	35.9
20	2.14	145				7.1	55.4	52.0
		197				0.3	4.8	10.5
		252				5.5	32.1	79.9
		306				12.7	48.0	137.5
		372	98.0			7.2	54.4	100.8
L. G.		68	97.4			5.5	101.4	221.5
25	2.20	139				7.1	77.4	176.7
		198				3.0	31.2	48.5
		252				10.9	85.5	84.2
		309				9.4	108.7	73.1
		369	98.2			8.5	49.4	117.9

* Each value represents a single determination. Typhoid vaccine was administered at the end of the first period. All subjects were premedicated with Aminopyrine. Abbreviations are as in Table I.

TABLE III

Mean plasma and urinary electrolyte values in sixteen control subjects and in sixteen subjects before and during the pyrogenic reaction *

Hours	Plasma		Urine	
	N_a *mEq./L.*	K *mEq./L.*	$U_{Na}V$ *μEq./min.*	U_kV *μEq./min.*
Control subjects				
0–1	138.0 ± 1.0	4.29 ± 0.13	103.3 ± 18.8	72.7 ± 8.1
2–3	139.5 ± 0.7	4.17 ± 0.14	153.0 ± 21.4	77.0 ± 6.0
Experimental (pyrogen) subjects				
0–1	136.0 ± 0.7	4.12 ± 0.05	91.6 ± 16.4	85.9 ± 11.8
2–3	136.7 ± 0.6	3.76 ± 0.05	119.0 ± 24.4	64.1 ± 3.7

* Each value is the mean and standard error of the mean for all subjects in the control and experimental groups, respectively. The time in hours is in reference to the beginning of the first collection period. The experimental subjects receiving typhoid vaccine are those listed in Tables I and II. The mean values for this group during the first hour represent resting values prior to an effect of the pyrogenic reaction. The 2- to 3-hour values are those obtained at the time of intrarenal hyperemia. Abbreviations are as shown in Table I.

ministration of the vaccine, at the time of renal hyperemia, sodium output did not change consistently. The mean excretion at this time exceeded resting levels (by more than 10 per cent) in 10 of 16 subjects and was less than or equal to these values in the remainder. The mean change for the entire group of 16 subjects during the third hour [4] was not significant when compared to resting values ($t = 1.55$; $< .50 > .10$) or when compared to the mean spontaneous changes observed over a similar time interval in control subjects ($t = 0.88$; $p < .50 > .10$) (Table III). No difference was noted between febrile and afebrile subjects. The sodium content of plasma did not change significantly ($t = 1.64$; $p < .50 > .10$) (Table III).

Potassium excretion decreased (20 to 46 per cent) one to two hours following administration of typhoid vaccine in nine of eleven subjects in whom renal hemodynamics were measured (U_kV, Table I). Potassium output increased in J. McN. and remained unchanged in S. P. at this time. Although the reduction in potassium excretion was usually accompanied by intrarenal vasoconstriction, this change occurred independently of alterations in glomerular filtration rate and in one instance (G. P.) occurred in the absence of a change in renal plasma flow. During hyperemia potassium output remained essentially constant except in J. P., in whom the excretion of this ion increased and in S. P. and G. R., in whom it diminished. A similar response was observed in five subjects in whom renal hemodynamics were not studied (Table II). Potassium output fell in three (H. H., D. B., and L. G.) of these subjects, remained unchanged in one (F. W.) and increased in one (W. L.) during the first two to three hours following administration of the vaccine. During the fourth and fifth hours the urinary excretion of this ion increased above resting levels in four of five studies. No difference was observed between febrile and afebrile subjects in either group of subjects.

The mean reduction in potassium output from resting levels in all 16 subjects during the first one to two hours of the pyrogenic reaction was of borderline significance ($t = 2.70$; $p < .02 > .01$). This change was not significant when compared to the control group ($t = 1.53$; $p < .50 > .10$). During hyperemia the mean potassium output did not differ significantly from resting levels ($t = 2.01$; $p < .10 > .05$) (Table III), nor was the mean difference significant when compared to spontaneous changes observed in control subjects ($t = 1.53$; $p < .50 > .10$) (Table III).

The concentration of potassium in plasma decreased during the pyrogenic reaction in 8 of 14 subjects (plasma electrolytes were not measured in two). In the remainder no change was noted. The mean reduction for all 14 subjects, 0.35 μEq. per L. (range, 0 to 0.80 μEq. per L.), was significant when compared with resting levels ($t = 7.0$; $p < .01$) (Table III), but not when compared

[4] This time was selected in order to analyze statistically the changes during hyperemia in all 16 subjects. The subjects listed in Table I were not studied beyond the third hour.

with the mean spontaneous change in control subjects studied during a simliar interval of time ($t = 1.97$; $p < .10 > .05$) (Table III).

DISCUSSION

In the present study no definite interrelation was demonstrated between urinary electrolyte excretion and the renal circulatory adjustments attending the pyrogenic reaction. During the initial vasoconstrictive phase of this reaction, when renal plasma flow and glomerular filtration rate were transiently reduced, sodium and potassium output were not altered systematically, nor was there a significant change in the excretion of these ions during the later and more sustained phase of intrarenal hyperemia.

Previous evidence of a relationship between renal hemodynamics and urinary sodium excretion has been obtained in a variety of experimental and clinical conditions which have in common intrarenal vasoconstriction (2, 4, 5). The mechanism by which sodium excretion is reduced under these conditions is not clear but changes in glomerular filtration rate (2, 7) and perhaps in the intrarenal distribution of blood (6) may play a role. Whether changes in filtration are necessary has not been established. It has been suggested that electrolyte reabsorption may be influenced by the rate of blood flow through the kidney (4, 5). Although the present study does not support this view, since sodium and potassium excretion remained unchanged during both renal vasoconstriction and hyperemia, further studies are needed in order to evaluate this problem in view of the many physiologic and metabolic alterations elicited during the pyrogenic reaction (12) which might influence electrolyte metabolism and reabsorption independently of renal hemodynamics. The results of this study cannot be interpreted with certainty in terms of the relationship between the renal circulation and tubular function. Whether an interrelation between electrolyte excretion and hyperemia exists under circumstances other than the pyrogenic reaction must be determined. During renal vasodilatation as elicited by intravenous l-hydrazinophthalazine, urinary sodium excretion is unaltered (13).

Although alterations in renal hemodynamics did not affect electrolyte excretion in the present study, the reduction in glomerular filtration rate during the vasoconstrictive phase of the pyrogenic reaction appeared to initiate a fall in urine volume, as has been demonstrated previously in man (7), and the urine became more concentrated with respect to sodium and potassium ions. That factors other than a change in filtration contributed to the reduced urine output is suggested by the fact that, once initiated, the antidiuresis tended to persist despite a return of glomerular filtration rate to control levels. These observations differ from those reported recently by Brandt, Ruskin, Zumoff, Castleman, and Zuckerman (14) who describe an increase in the free water clearance of dogs following administration of a bacterial pyrogen derived from pseudomonas.

SUMMARY

A study was made of the effect of the pyrogenic reaction as elicited by triple typhoid vaccine on the urinary excretion of sodium and potassium in 16 normal human subjects. No systematic change in the excretion of these ions was observed in febrile or afebrile subjects during either the early, transient period of renal vasoconstriction or the late, sustained period of renal hyperemia. A reduction in urine volume occurred during the vasoconstrictive phase in association with a decrement in glomerular filtration rate and the urine became more concentrated with respect to sodium and potassium ions.

ACKNOWLEDGMENT

The technical assistance of Miss Marion Brazel and Mrs. Frances Kennedy is gratefully acknowledged.

REFERENCES

1. Lathem, W., Urinary excretion of sodium and potassium during the pyrogenic reaction. J. Clin. Invest., 1954, **33**, 951.
2. Selkurt, E. E., Sodium excretion by the mammalian kidney. Physiol. Rev., 1954, **34**, 287.
3. Smythe, C. McC., Nickel, J. F., and Bradley, S. E., The effect of epinephrine (USP), *l*-epinephrine, and *l*-norepinephrine on glomerular filtration rate, renal plasma flow, and the urinary excretion of sodium, potassium, and water in normal man. J. Clin. Invest., 1952, **31**, 499.
4. Elkinton, J. R., Crosley, A. P., Jr., Barker, H. G., and Clark, J. K., Alterations in renal hemodynamics and excretion of electrolytes. Federation Proc., 1950, **9**, 37.

5. Nickel, J. F., Smythe, C. McC., Papper, E. M., and Bradley, S. E., A study of the mode of action of the adrenal medullary hormones on sodium, potassium and water excretion in man. J. Clin. Invest., 1954, **33**, 1687.
6. Goodyer, A. V. N., and Jaeger, C. A., Renal response to nonshocking hemorrhage. Role of the autonomic nervous system and of the renal circulation. Am. J. Physiol., 1955, **180**, 69.
7. Smith, H. W., The Kidney: Structure and Function in Health and Disease. New York, Oxford Univ. Press, 1951.
8. Harrison, H. E., A modification of the diphenylamine method for determination of inulin. Proc. Soc. Exper. Biol. & Med., 1942, **49**, 111.
9. Smith, H. W., Finkelstein, N., Aliminosa, L., Crawford, B., and Graber, M., The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J. Clin. Invest., 1945, **24**, 388.
10. Bradley, S. E., Chasis, H., Goldring, W., and Smith, H. W., Hemodynamic alterations in normotensive and hypertensive subjects during the pyrogenic reaction. J. Clin. Invest., 1945, **24**, 749.
11. Chasis, H., Ranges, H. A., Goldring, W., and Smith, H. W., The control of renal blood flow and glomerular filtration in normal man. J. Clin. Invest., 1938, **17**, 683.
12. Bennett, I. L., Jr., and Beeson, P. B., The properties and biologic effects of bacterial pyrogens. Medicine, 1950, **29**, 365.
13. Judson, W. E., Hollander, W., Friedman, I. H., Hatcher, J. D., and Halperin, M. H., Studies on congestive failure: The effects of exercise, salt loading and of intravenous apresoline on cardiorenal hemodynamics and the excretion of sodium and water. J. Clin. Invest., 1953, **32**, 579.
14. Brandt, J. L., Ruskin, H. D., Zumoff, B., Castleman, L., and Zuckerman, S., Inhibition of renal tubular responsiveness to antidiuretic hormone by pyrogens. Proc. Soc. Exper. Biol. & Med., 1955, **88**, 451.

STUDIES ON THE PHYSIOLOGY OF HUNGER. I. THE EFFECT OF INTRAVENOUS ADMINISTRATION OF GLUCOSE ON GASTRIC HUNGER CONTRACTIONS IN MAN[1]

By ALBERT J. STUNKARD and HAROLD G. WOLFF with the technical assistance of ALBA PLESCIA

(From the Study Program in Human Health and the Ecology of Man, Departments of Medicine and Psychiatry, New York Hospital-Cornell Medical Center, New York, N. Y.)

(Submitted for publication December 27, 1955; accepted May 17, 1956)

The introduction of measures for the recording of gastric hunger contractions half a century ago laid the foundations for the modern study of appetite and hunger (1, 2). The finding that intravenous administration of glucose could abolish these contractions in fasting dogs led Bulatao and Carlson to postulate that "(lack of) availability of carbohydrates for utilization . . . is an important factor in the genesis of the gastric hunger contractions" (3). Further evidence for this hypothesis was not forthcoming, and later reports of the effects of glucose administration on gastric motility have been inconsistent, and even in conflict (4–6).

Recently this problem has been reopened by Mayer's proposal of a "glucostatic" theory of the regulation of food intake which ascribes hunger to a depletion of the carbohydrate stores of the body, and satiety to the replenishment of these stores (7, 8). According to this theory the status of the carbohydrate stores is signalled to the hypothalamic food regulatory centers by the rate at which glucose is utilized.

Since experiments of the type performed by Bulatao and Carlson should provide a test of the glucostatic theory, it seemed important to repeat these studies in man to determine with greater assurance the effects of glucose on gastric hunger contractions. The peripheral removal of glucose provides an index of the metabolic events accompanying such injection. Accordingly, peripheral capillary-venous glucose differences were determined at intervals throughout the experiment, and these values were correlated with the effects of glucose injection on gastric hunger contractions.

[1] This work was supported in part by the Research and Development Division, Office of the Surgeon General, Department of the Army, Contract No. DA-49-007-MD-524.

METHODS AND MATERIALS

Twenty-three adult persons served as subjects for this investigation, 9 on more than one occasion. Three were healthy volunteers, 20 were patients from the general medical clinic of the New York Hospital, 5 with a diagnosis of diabetes mellitus, 15 with diagnoses as listed in Tables I and II.

Each subject was studied in the morning after an overnight fast. At the beginning of the experiment a Levine tube with attached gastric balloon was inserted into the stomach. The balloon was inflated to a pressure of 10 cm. of water and the tube was withdrawn until resistance was encountered at the cardia. The tube was then taped into place at the nose. Gastric contractions were recorded on a kymograph by means of a water manometer. An intravenous infusion of isotonic sodium chloride was begun and base-line capillary and venous blood samples were drawn. Carefully standardized control periods of from three to five hours allowed assessment of the subject's pattern of gastric activity as well as the effect on gastric motility of a rapid (3-minute) injection of 70 ml. of saline through the infusion tubing. In only one subject was such saline injection followed by inhibition of gastric contractions; he was excluded from the study.

During a period of vigorous gastric contractions, 50 ml. of a 50 per cent solution of glucose were administered through the infusion tubing over a period of 3 minutes. In half the cases this was associated with transient discomfort in the area above the needle; the remaining subjects were either unaware of the injection or experienced no discomfort. There was no correlation between such reactions and subsequent inhibition of gastric hunger contractions.

Venous and capillary blood samples were obtained according to two different schedules; at 10, 30 and 50 minutes, and at 20, 40 and 60 minutes following completion of the glucose injection. To conserve space all of these values are not recorded in the tables. Venous blood was drawn with minimal stasis from an antecubital vein, and capillary blood was obtained almost simultaneously by direct pipetting from a finger tip of the same extremity after cutaneous puncture. Capillary blood has been shown to have the same glucose content as arterial blood (9–11).

Glucose in whole blood was determined by the method of Somogyi as modified by Nelson (12–14). Determinations were done in duplicate on 0.2-ml. samples. Stand-

TABLE I

Subjects in whom gastric contractions ceased after glucose injection

Subject	Age	Sex	Diagnosis	Fasting glucose values: Capillary / Venous	Fasting glucose values: Difference	Glucose values at 10 minutes: Capillary / Venous	Glucose values at 10 minutes: Difference	Glucose values at 20 minutes: Capillary / Venous	Glucose values at 20 minutes: Difference	Glucose values when contractions returned: Capillary / Venous	Glucose values when contractions returned: Difference	Time when contractions returned (*minutes*)	Glucose values at 47 minutes in subjects without return of contractions: Capillary / Venous	Glucose values at 47 minutes in subjects without return of contractions: Difference
D. W. (1)	25	M	No disease	86 / 83.5	2.5			147 / 119.5	27.5	83.5 / 74.5	9	57		
I. L.	28	M	Anxiety reaction	82 / 80	2	191 / 157	34	155 / 128.5	26.5	79 / 71	8	45		
G. S.	43	M	No disease	62.5 / 57	5.5			189.5 / 168.5	21	135.5 / 126	9.5	58		
G. S.	43	M	No disease	88.5 / 83	5.5	230 / 213.5	16.5	208 / 189	19				142 / 125	17
Ma. B. (3)	56	F	Paranoid reaction	71 / 70	1	211 / 194	17	193.5 / 175.5	18				142.5 / 127.5	15
A. L. (2)	33	F	Spastic colon	84 / 80	4	233 / 218.5	14.5	208.5 / 194	14.5				130 / 119	11
R. A.	34	M	Obesity*	52 / 45	7			146.5 / 132	14.5	106 / 108	−2	59		
R. L. (2)	49	F	Neuro-dermatitis	64 / 60	4			185.5 / 171.5	14	135 / 133	2	45		
R. R.	50	F	Obesity	94 / 87.5	6.5	226 / 211	15	207.5 / 198.5	9	149.5 / 147	2.5	54		
E. S.	38	F	Obesity	77 / 77	0			184.5 / 175.5	9	165 / 151	14	27		

* "Obesity"—More than 20 per cent over standard weight.

TABLE I—*Continued*

Subject	Age	Sex	Diagnosis	Fasting glucose values: Capillary / Venous	Difference	Glucose values at 10 minutes: Capillary / Venous	Difference	Glucose values at 20 minutes: Capillary / Venous	Difference	Glucose values when contractions returned: Capillary / Venous	Difference	Time when contractions returned (*minutes*)	Glucose values at 47 minutes in subjects without return of contractions: Capillary / Venous	Difference
R. Z.	36	F	Obesity	98 / 92.5	5.5	185.5 / 175	10.5	170.5 / 162.5	8	142.5 / 139	3.5	36		
N. B.	59	F	Diabetes mellitus, Obesity	100 / 96.5	3.5			213 / 205	8				178.5 / 169.5	9
A. L. (3)	33	F	Spastic colon	80 / 72.5	7.5	231 / 221	10	208.5 / 202	6.5				157 / 148	9
P. H. (1)	56	M	Diabetes mellitus	211 / 208.5	2.5	318.5 / 308.5	10	302.5 / 297.5	5	271 / 275	−4	53		
I. G. (1)	51	F	Anxiety reaction, Obesity	73 / 77	−4			170 / 165.5	4.5	164 / 160	4	27		
J. D.	36	F	Spastic colon	75.5 / 75	0.5	227.5 / 224	3.5	204.5 / 204	0.5	136 / 139	−3	52		
I. G. (2)	51	F	Anxiety reaction, Obesity	70 / 72	−2	201.5 / 199	2.5	185 / 185	0	154 / 152.5	1.5	41		
H. H.	61	M	Diabetes mellitus, Obesity	111 / 107.5	3.5	205.5 / 211	−5.5	201 / 203.5	−2.5	170 / 170.5	−0.5	58		
T. B.	50	F	Osteo-arthritis	80 / 78	2			188 / 191	−3	153 / 147.5	5.5	46		
Mean and Standard Deviation				87.3±33.1 / 84.3±33.2	3.0±3.0	223.6±35.6 / 212.0±38.3	11.6±10.2	193.1±33.7 / 182.5±38.1	10.5±8.3	146.0±45.9 / 142.4±48.2	3.6±5.2	47.0±11.9	150.0±18.6 / 137.8±20.8	12.2±3.6

TABLE II

Subjects in whom gastric contractions persisted after glucose injection

Subject	Age	Sex	Diagnosis	Fasting glucose values: Capillary / Venous	Fasting glucose values: Difference	Glucose values at 10 minutes: Capillary / Venous	Glucose values at 10 minutes: Difference	Glucose values at 20 minutes: Capillary / Venous	Glucose values at 20 minutes: Difference
A. L. (1)	33	F	Spastic colon	79 / 74	5			204.5 / 184.5	20
L. T.	45	M	(Treated) Anemia	88 / 84.5	3.5	191.5 / 178.5	13	179.5 / 168	11.5
P. H. (2)	56	M	Diabetes mellitus	111 / 111	0			177 / 166	11
D. W. (2)	25	M	No disease	85 / 82	3	178.5 / 171	7.5	165.5 / 155	10.5
M. G.	25	M	No disease	67.5 / 68.5	−1			166.5 / 160	6.5
P. H. (3)	56	M	Diabetes mellitus	77 / 67	10			148.5 / 142.5	6
M. B. (1)	26	F	Diabetes mellitus	186 / 176	10			259 / 255.5	3.5
V. N.	59	F	Osteo-arthritis	94 / 92.5	1.5			213.5 / 210	3.5
Ma. B. (2)	56	F	Paranoid reaction	79 / 77.5	1.5	192 / 190	2	172 / 169	3
M. M. (1)	60	M	Diabetes mellitus	109 / 104	5			248 / 245	3
M. B. (2)	27	F	Diabetes mellitus	219 / 217	2	281 / 278	3	271 / 268	3
I. V.	27	F	(Treated) Hypothy-roidism	85 / 84	1			184.5 / 187	−2.5
R. L. (1)	49	F	Neuro-dermatitis	73 / 70	3			161 / 165	−4
V. S.	43	F	Depressive reaction	80 / 81	−1	188.5 / 197.5	−9	176 / 181.5	−5.5
M. M. (2)	60	M	Diabetes mellitus	— / (117)				236 / 246	−10
Ma. B. (1)	55	F	Paranoid reaction +Cortisone	78 / 77	1	207 / 217	−10	189 / 199.5	−10.5
Mean and Standard Deviation				100.7±43.4 / 97.7±42.5	3.0±3.4	206.4±36.4 / 205.3±39.0	1.1±9.1	197.0±37.7 / 194.0±39.5	3.1±8.2

ard error of the mean of 30 consecutive duplicate determinations was 1.48 mg. per cent for values below 120 per cent, and 1.76 mg. per cent for values above 120 mg. per cent. In one-third of the experiments the determination of known amounts of glucose added to whole blood was performed with an average recovery of 94 per cent.

RESULTS

On 19 of 40 occasions intravenous administration of 50 ml. of a 50 per cent solution of glucose was followed within 5 minutes by cessation of gastric hunger contractions. This "contractions inhibited" group is listed in Table I together with age, sex, diagnosis and glucose values of its members. In 14 of these 19 experiments there was a return of contractions 47 ± 12 minutes after their inhibition. In no case had previous rapid intravenous injection of 70 ml. of isotonic saline, venipunctures, or finger punctures had any effect on gastric motility.

On 16 occasions gastric hunger contractions persisted for at least 20 minutes following glucose administration. The data for the "contractions persist" group are listed in Table II. Five experiments in which contractions ceased between 5 and 20 minutes after glucose injection were considered inconclusive and are not included in either group.

Fasting capillary-venous glucose differences

A measure of the reproducibility of the capillary-venous glucose differences is obtained from the fasting values noted in Tables I and II. Thus,

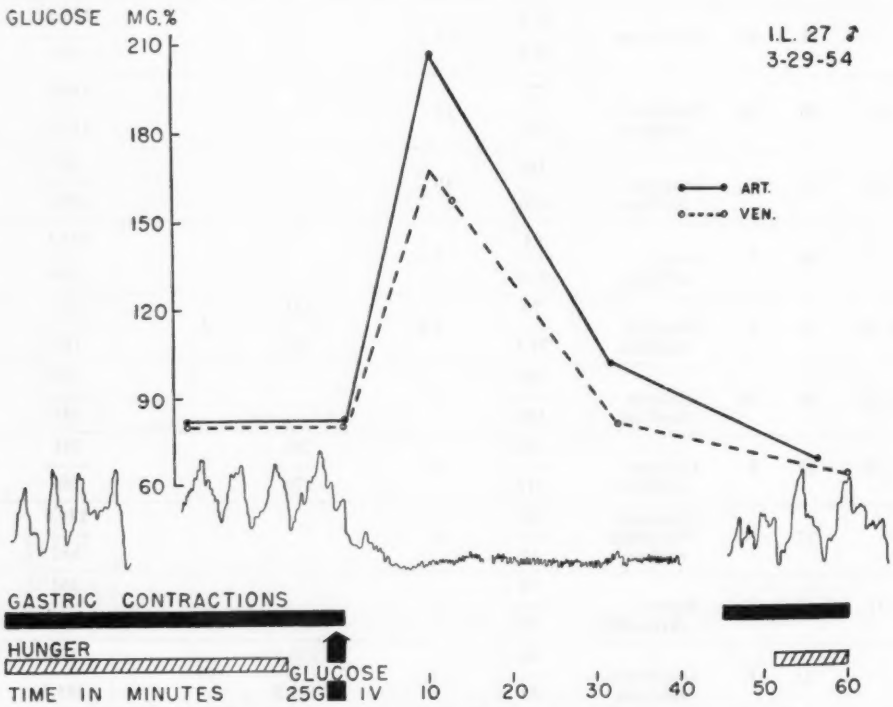


FIG. 1. (SUBJECT I. L.) CESSATION OF GASTRIC HUNGER CONTRACTIONS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 50 ML. OF A 50 PER CENT SOLUTION OF GLUCOSE

Ten minutes after the injection the rise in blood glucose is associated with a capillary-venous difference of 34 mg. per cent. When blood glucose levels fall, this difference decreases rapidly, reaching a value of 8 mg. per cent at the time gastric hunger contractions return, 45 minutes after injection.

The sample tracings are from a kymograph revolving at a speed of one inch per minute. The black bar represents the actual time relations of the contractions to blood glucose levels and to the subjective experience of hunger which is indicated by the cross-hatched bar.

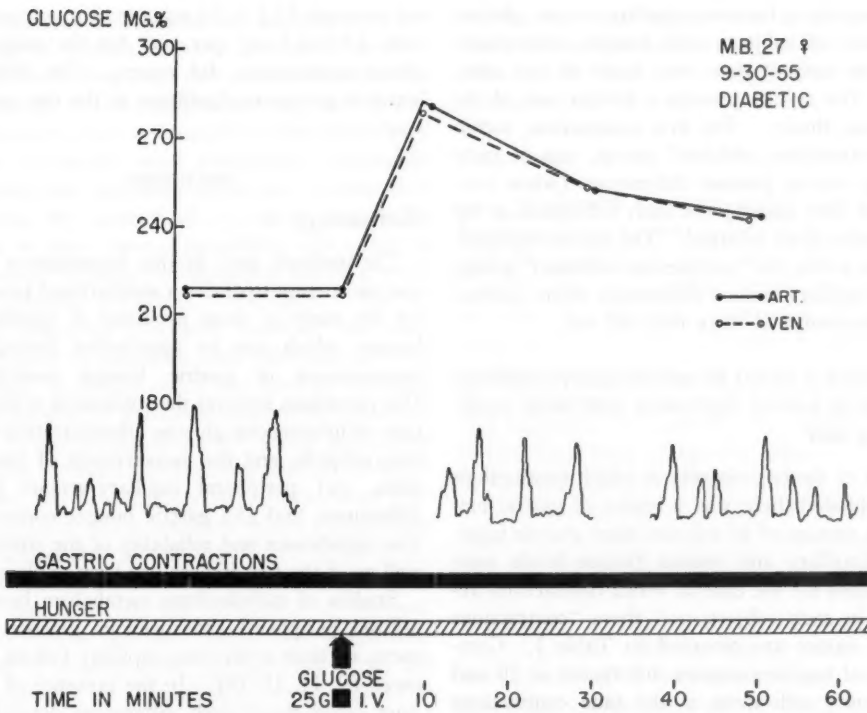


FIG. 2. (SUBJECT M. B.) PERSISTENCE OF GASTRIC HUNGER CONTRACTIONS IN A DIABETIC SUBJECT FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 50 ML. OF A 50 PER CENT SOLUTION OF GLUCOSE

Although blood glucose levels rise considerably over the fasting hyperglycemia, the capillary-venous difference remains small.

although capillary and venous values are somewhat higher in the "contractions persist" group because of its higher percentage of diabetic subjects, the capillary-venous differences of the two groups do not differ. Their small value (3.0 ± 3.0 mg. per cent [2] for the "contractions inhibited" group and 3.0 ± 3.4 mg. per cent for the "contractions persist" group) indicates negligible peripheral removal of glucose in the fasting state, and corresponds closely to the findings of other investigators (9–11, 15).

Early capillary-venous glucose differences (10 and 20 minutes following glucose injection)

Higher capillary-venous glucose differences occur in the "contractions inhibited" group than in the "contractions persist" group at both 10 and 20 minutes even though there is considerable overlap. The means of the capillary-venous glucose differences of the two groups at 10 minutes are 11.6 ± 10.2 mg. per cent for the "contractions inhibited" experiments, and 1.1 ± 9.1 mg. per cent for the "contractions persist" experiments. At 20 minutes the mean capillary-venous difference of the "contractions inhibited" group is 10.5 ± 8.3 mg. per cent, while that of the "contractions persist" group is 3.1 ± 8.2 mg. per cent. Differences between the two groups are significant at the 5 per cent level.[3] Graphic illustration of these findings is provided by Figures 1 and 2.

[2] Standard deviation.

[3] In each comparison in this report capillary-venous glucose differences ranked in order of their magnitude were subjected to statistical analysis by means of the rank "t" test. This instrument was considered most appropriate for the material since it requires no assumption of a normal distribution (16).

A comparison between capillary-venous glucose differences of subjects with hunger contractions and those without them was made at two other times. The results provide a further test of the glucostatic theory. The first comparison, within the "contractions inhibited" group, was of early capillary-venous glucose differences (when contractions were absent) and such differences at the time contractions returned. The second comparison, also within the "contractions inhibited" group, was of capillary-venous differences when contractions returned, and when they did not.

Comparison of early (10- and 20-minute) capillary-venous glucose differences with those occurring later

In 14 of the experiments in which contractions were inhibited there was a return of gastric motility an average of 47 minutes after glucose injection. Capillary and venous glucose levels were interpolated for the time at which contractions returned in each subject, and these "contractions return" values are recorded in Table I. Comparison of capillary-venous differences at 10 and 20 minutes with those at the time contractions returned reveals that the latter values are usually lower. The means are 10.0 ± 12.5 mg. per cent at 10 minutes and 9.6 ± 10.0 mg. per cent at 20 minutes. The means of the "contractions return" group are 1.4 ± 4.1 mg. per cent for the 7 subjects whose 10-minute values are available, and 3.6 ± 5.2 mg. per cent for the group as a whole. The difference between groups is not significant at the 5 per cent level ($P = 0.11$ and 0.12).

Comparison of capillary-venous glucose differences when contractions returned and when they did not

In 5 of the 19 experiments in which contractions were inhibited they did not return during the period of observation. Table I lists the capillary-venous glucose differences 47 minutes after glucose injection for these 5 experiments. These values are considerably larger than the capillary-venous differences at the time contractions returned (an average of 47 minutes after injection) in the other 14 experiments of the "contractions inhibited" group. The mean of the capillary-venous differences for the group in which contractions did not return is 12.2 ± 3.6 mg. per cent, as compared with 3.6 ± 5.2 mg. per cent for the subjects in whom contractions did return. The difference between groups is significant at the one per cent level.

DISCUSSION

Methodology

The methods used in this investigation represent an attempt to devise a standardized procedure for the study of those problems of appetite and hunger which can be approached through the measurement of gastric hunger contractions. This procedure involves the application of the stimulus of intravenous glucose administration to human subjects, and the measurement of two variables, (a) peripheral capillary-venous glucose differences, and (b) gastric hunger contractions. The significance and reliability of the stimulus as well as of the two indices has been explored.

Studies of carbohydrate metabolism have long utilized peripheral arteriovenous glucose differences, or their equivalent, capillary-venous differences (9, 11, 17–19). In the presence of a constant blood flow,[4] such differences depend upon the rate at which glucose is removed by the tissues, and these removal rates are closely related to the general level of carbohydrate stores. Thus, during fasting capillary-venous glucose differences are very small, and they increase in magnitude with carbohydrate repletion, notably during a glucose tolerance test, or following a meal. The relevance of these findings to the present study has been established by Van Itallie, Beaudoin, and Mayer who demonstrated an excellent temporal correlation between such variations in capillary-venous glucose differences and naturally occurring states of hunger and satiety (21).

Recording of gastric hunger contractions is a convenient and objective measure of the hunger state. It has, however, certain limitations. In the

[4] The assumption of a constant blood flow under the conditions of this investigation is supported by a recent study which showed that the intravenous injection of enough 40 per cent glucose solution to raise the blood sugar to 300 mg. per cent (higher than that recorded here) had no effect on the skin temperature of the fingers, hands or forearms (20). It seems reasonable that the far more stable blood flow of the muscles of this area would not be affected.

first place, the contractions, like the subjective experience of hunger, are not constantly present even in the fasting state, but occur periodically on the average of one-half out of every two hours. Furthermore, they are subject to inhibition by a variety of stimuli, most prominently unpleasant sensations and emotions. It is thus possible that, following the injection of any agent, nonspecific stimuli or their normal periodicity will result in a cessation of hunger contractions, and this could be erroneously attributed to the agent. The injection procedure previously described was used in an effort to minimize such inhibition of contractions. A further effort was made to minimize the importance of nonspecific stimuli by including in the "contractions inhibited" group only those instances in which contractions ceased within 5 minutes, and by confining the "contractions persist" group to those subjects whose contractions lasted longer than 20 minutes.

The administration of glucose by the intravenous route adds further difficulties of method. This technique provides a rapid and predictable method of raising blood glucose levels, but the hyperglycemia so obtained may not correspond to that following the ingestion of food or even of glucose. In the first place the injected glucose is strongly hypertonic and in this respect dissimilar to that obtained by intestinal absorption. This very hypertonicity may have inhibitory effects on gastric hunger contractions. In the second place, the glucose enters the body through a peripheral route, differing from its absorption through the intestinal wall following oral administration. Thirdly, the rapid rise in blood glucose levels is distinctly unphysiological. Finally, the glucose so administered may be metabolized in a different manner from that present in the blood under normal circumstances. Scow and Cornfield, for example, have demonstrated that orally administered glucose is removed from the blood three times as rapidly as glucose injected intravenously (22). The small size of the capillary-venous differences reported here is additional evidence for slower removal following intravenous injection of glucose; they are far smaller than those reported following oral administration (11).[5] Furthermore, this occurs contrary to predictions based on the law of mass action, for the levels following injection are far higher than those following oral administration.

Significance

A relationship between gastric hunger contractions, hunger and carbohydrate metabolism was first postulated more than 30 years ago. Certain observations from clinical medicine support this view; depletion of available carbohydrate offers an explanation of hunger in such diverse conditions as starvation, unregulated diabetes mellitus, cortisone therapy and insulin hypoglycemia. The extensive experimental data relating to this problem have been reviewed by Mayer (7, 24) and by Grossman (25).

Certain observations of earlier workers indicated that it might be fruitful to investigate further the relation between glucose and gastric hunger contractions. As has been noted, Bulatao and Carlson reported that intravenous administration of glucose abolished hunger contractions in normal, but not in pancreatectomized, dogs. Independent confirmation of this finding was reported (4), but later, careful work has cast doubt upon its validity (5, 6). On one matter there has been unanimity: all reports have agreed that glucose abolishes gastric hunger contractions which have been induced by insulin hypoglycemia (4–6, 26, 27). Since insulin administration presumably increases the rate at which glucose is utilized (18, 28), this observation suggests that it is the utilization rate which determines the effect of intravenous glucose on gastric motility. None of these studies, however, reported an attempt to measure such utilization.

The current study is apparently the first attempt to combine observation of the effects of glucose injection on gastric motility with an index of glucose utilization. This has made it possible to correlate capillary-venous glucose differences and gastric hunger contractions in three different con-

[5] An alternative explanation of the small capillary-venous differences is suggested by the observation that, following intravenous injection of a non-metabolizable sugar, diffusion from the extravascular compartment back into the blood stream results in negative capillary-venous differences (23). Some such diffusion phenomenon may occur even following glucose administration to normal subjects, and seems the most reasonable explanation of the negative capillary-venous differences observed in the diabetic subjects.

texts. Although none of these correlations is conclusive, and one is not even statistically significant, each points to a tendency for gastric quiescence to be associated with higher capillary-venous glucose differences than are found in subjects in whom hunger contractions persisted.

If the phenomena of hunger and satiety are distinguished as current theory demands (*29*), these findings are relevant chiefly to the latter function. They suggest that metabolic events accompanying increased peripheral removal of glucose may play a role in the normal satiety mechanism. They do not support the view that depletion of carbohydrate stores of the order produced by an overnight fast is the sole, or even a sufficient, stimulus for hunger. Fasting subjects (with negligible capillary-venous glucose differences) spent many hours during the control periods with neither the subjective experience of hunger nor the presence of gastric hunger contractions. This does not contradict the position that carbohydrate depletion is a necessary precondition of hunger. The available evidence would support this view.

The study provides evidence that a fall in blood glucose, even at hyperglycemic levels, may induce hunger contractions. Although there is general agreement that hypoglycemia induced by insulin is associated with gastric hunger contractions, it has only once been suggested that glucose administration itself can be a stimulus to such contractions, and this only during the hypoglycemic phase (5). However, in the group with a rapid shrinking of the capillary-venous glucose differences, the return of contractions an average of 47 minutes after their abolition is sooner than would be predicted from the previous spontaneous gastric activity of these subjects.

The low order of statistical significance attached to the findings of this study despite a relatively large number of observations may indicate that metabolic events accompanying increased peripheral capillary-venous glucose differences play an unimportant role in the inhibition of gastric hunger contractions. On the other hand, limitations of method could give the same result. Foremost among these limitations has been the inability to reproduce the pattern of glycemia, including the large capillary-venous glucose differences, which normally follows the ingestion of food. If the present findings are correctly interpreted, an agent capable of such effects should abolish gastric hunger contractions even at lower glucose levels than those which follow its intravenous administration. It has recently been shown that glucagon has this effect upon blood glucose (30), and preliminary study has indicated that its administration predictably abolishes gastric hunger contractions (31). Details of these findings will be the subject of a further report.

SUMMARY AND CONCLUSIONS

1. Intravenous administration of 50 ml. of a 50 per cent solution of glucose was followed within 5 minutes by the cessation of gastric hunger contractions in 19 of 40 experiments on a group of diabetic and non-diabetic human subjects.

2. At 10 and 20 minutes following injection, the peripheral capillary-venous glucose differences in the group of 19 subjects in which contractions were inhibited were significantly higher than those of the group in which contractions persisted.

3. In 14 of the 19 experiments in which contractions were inhibited, they returned at an average of 47 minutes after injection. Capillary-venous glucose differences at 10 and 20 minutes after injection were usually higher than those at the time contractions returned, but this difference was not significant at the 5 per cent level.

4. Capillary-venous glucose differences in the 5 experiments in which contractions remained inhibited were significantly higher than those in the 14 experiments in which they returned.

5. These findings support the view that metabolic events accompanying *increased* peripheral removal of glucose have an inhibiting effect on gastric hunger contractions. Events associated with *decreased* removal, on the other hand, are not a sufficient stimulus for the production of gastric hunger contractions, although they appear to be a necessary precondition.

REFERENCES

1. Cannon, W. B., and Washburn, A. L., An explanation of hunger. Am. J. Physiol., 1912, **29**, 441.
2. Carlson, A. J., The control of hunger in health and disease. Chicago, Univ. Chicago Press, 1916.
3. Bulatao, E., and Carlson, A. J., Contributions to the physiology of the stomach. Influence of experimental changes in blood sugar level on gastric

hunger contractions. Am. J. Physiol., 1924, **69**, 107.
4. Templeton, R. D., and Quigley, J. P., The action of insulin on the motility of the gastrointestinal tract. II. Action on the Heidenhain pouch. Am. J. Physiol., 1930, **91**, 467.
5. Quigley, J. P., and Hallaran, W. R., The independence of spontaneous gastro-intestinal motility and blood sugar levels. Am. J. Physiol., 1932, **100**, 102.
6. Mulinos, M. G., The gastric hunger mechanism. IV. The influence of experimental alterations in blood sugar concentration on the gastric hunger contractions. Am. J. Physiol., 1933, **104**, 371.
7. Mayer, J., Genetic, traumatic and environmental factors in the etiology of obesity. Physiol. Rev., 1953, **33**, 472.
8. Mayer, J., Glucostatic mechanism of regulation of food intake. New England J. Med., 1953, **249**, 13.
9. Foster, G. L., Studies on carbohydrate metabolism. I. Some comparisons of blood sugar concentration in venous blood and in finger blood. J. Biol. Chem., 1923, **55**, 291.
10. Langner, P. H., Jr., and Fies, H. L., Blood sugar values of blood obtained simultaneously from the radial artery, antecubital vein, and the finger. Am. J. Clin. Path., 1942, **12**, 559.
11. Somogyi, M., Studies of arteriovenous differences in blood sugar. I. Effect of alimentary hyperglycemia on the rate of extrahepatic glucose assimilation. J. Biol. Chem., 1948, **174**, 189.
12. Somogyi, M., Determination of blood sugar. J. Biol. Chem., 1945, **160**, 69.
13. Somogyi, M., A new reagent for the determination of sugars. J. Biol. Chem., 1945, **160**, 61.
14. Nelson, N., A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 1944, **153**, 375.
15. Bell, D. M., Femoral arteriovenous sugar differences in fasting human beings. J. Lab. & Clin. Med., 1952, **40**, 337.
16. Bross, I. D. J., Is there an increased risk? Federation Proc., 1954, **13**, 815.
17. Bang, I., Der Blutzucker, 1913, Wiesbaden, Bergmann.
18. Bell, D. M., and Burns, T., Effect on femoral a-v glucose difference of insulin injected into an antecubital vein and into a femoral artery. J. Clin. Invest., 1952, **31**, 717.
19. Himsworth, H. P., The mechanism of diabetes mellitus. Lancet, 1939, **2**, 1, 65, 118, and 171.
20. Törnblom, N., and Hellman, S., On the mechanism of diabetes in obesity. Studies on arteriovenous glucose difference in normals and in obese diabetics. Acta med. Scandinav., 1953, **147**, 331.
21. Van Itallie, T. B., Beaudoin, R., and Mayer, J., Arteriovenous glucose differences, metabolic hypoglycemia and food intake in man. Am. J. Clin. Nutrition, 1953, **1**, 208.
22. Scow, R. O., and Cornfield, J., Quantitative relations between the oral and intravenous glucose tolerance curves. Am. J. Physiol., 1954, **179**, 435.
23. Van Itallie, T. B., Morgan, M. C., and Dotti, L. B., Peripheral diffusion characteristics of sucrose in man. Federation Proc., 1954, **13**, 156.
24. Mayer, J., Regulation of energy intake and the body weight: The glucostatic theory and the lipostatic hypothesis. Ann. New York Acad. Sc., 1955, **63**, 15.
25. Grossman, M. I., Integration of current views on the regulation of hunger and appetite. Ann. New York Acad. Sc., 1955, **63**, 76.
26. Quigley, J. P., and Templeton, R. D., Action of insulin on the motility of the gastrointestinal tract. III. a. Action on the pyloric pouch. b. Action on the stomach following double splanchnicotomy. Am. J. Physiol., 1930, **91**, 475.
27. Regan, J. F., The action of insulin on the motility of the empty stomach. Am. J. Physiol., 1933, **104**, 90.
28. Stadie, W. C., and Zapp, J. A., Jr., The effect of insulin upon the synthesis of glycogen by rat diaphragm in vitro. J. Biol. Chem., 1947, **170**, 55.
29. Brobeck, J. R., Neural regulation of food intake. Ann. New York Acad. Sc., 1955, **63**, 44.
30. Van Itallie, T. B., Morgan, M. C., and Dotti, L. B., Effect of glucagon on peripheral utilization of glucose in man. J. Clin. Endocrinol. & Metab., 1955, **15**, 28.
31. Stunkard, A. J., Van Itallie, T. B., and Reis, B. B., The mechanism of satiety: Effect of glucagon on gastric hunger contractions in man. Proc. Soc. Exper. Biol. & Med., 1955, **89**, 258.

DETERMINATION OF THE LIFE SPAN OF HUMAN BLOOD PLATELETS USING LABELLED DIISOPROPYLFLUOROPHOSPHONATE

By C. H. W. LEEKSMA and J. A. COHEN

(From the Department of Metabolic Diseases, University of Leyden and the Med. Biol. Lab. of the National Defense Research Council, T.N.O., Rijswijk, Holland)

(Submitted for publication January 20, 1956; accepted May 10, 1956)

Recently we have reported a method for the determination of platelet survival time using diisopropylfluorophosphonate (DFP) as the labelling agent (1). The present communication presents full details of this method.

Hitherto, no satisfactory method for the determination of platelet survival time has been described. Most data on this subject have been obtained by means of transfusion experiments (2–5). In these experiments the survival time of platelets is determined after transfusion of blood or platelet concentrates of normal and polycythemic donors into patients with aplastic anemia and other diseases where this determination was possible because of absence or morphological differences of the platelets of the recipient. In most cases a survival time of 3 to 6 days has been found. In some cases however a small part of the transfused platelets is found to be present in the circulation of the recipient 7 to 8 days after transfusion (5, 6). However, the results obtained in these studies are subject to considerable criticism since the platelets are damaged during the process of blood collection, concentration, and transfusion. The survival time of platelets is therefore partly determined by the technique used (4). Though several investigators have attempted to label platelets with radioactive isotopes (7–14), no technique has been provided that can be considered satisfactory with regard to the determination of survival time.

In a previous communication, Cohen and Warringa (15) reported the use of P^{32} labelled diisopropylfluorophosphonate (DFP^{32}) in the determination of the life span of erythrocytes. This method is based on the irreversible combination of DFP^{32} with esterases present in the red cell membrane. A mean red cell life span of 116 and 129 days, respectively, was found in two normal subjects. These values are in accordance with those reported in the literature.

Since thrombocytes have also been shown to contain esterases (16) the same method suggested itself as a way of measuring the life span of these cells. It seemed reasonable to assume that conditions similar to those described for red cells would obtain; viz., that the reaction of DFP^{32} with the proteins of platelets would be irreversible and that metabolites of DFP^{32} would be liberated only with the ultimate destruction of labelled cells. Shortly after the administration of DFP^{32} the DFP sensitive enzymes combine irreversibly and DFP is not linked to newly formed proteins since active DFP is not available. DFP administered intramuscularly is absorbed rapidly, and disappears from the circulation within a short period. Moreover the metabolites of DFP have been shown not to be incorporated in newly formed proteins.

METHODS

The labelled diisopropylfluorophosphonate (DFP^{32}) used was synthetized by R. A. Oosterbaan by a method which has been previously reported (17). DFP^{32} was administered intramuscularly in oil as described in an earlier report (15). Measurements of platelet-bound radioactivity were made at various intervals after the injection of the DFP^{32}. These studies were conducted on 5 patients without hematologic abnormalities (A, B, C, D, and G), three patients with polycythemia vera (F, M, and N) one patient with chronic myeloid leukemia (E), two postoperative patients (H and I), one patient with persistent thrombocytosis following splenectomy (K) and one patient with thrombocytopenic purpura (L) who had a remission following ACTH treatment. Data on these patients, the dose of DFP and the amount of radioactivity administered are presented in Table I.

Isolation of platelets. Forty-five to fifty-ml. blood samples are drawn from the antecubital vein into a siliconized tube and mixed with 5 ml. 1 per cent sodium ethylenediamine tetra-acetate in 0.9 per cent sodium chloride. The blood is transferred into siliconized centrifuge tubes and spun for ten minutes applying a force of 80 G. The supernatant platelet-rich plasma is removed with a siliconized pipette. Generally not all of the plasma can be removed without disturbing the buffy coat; hence a constant loss of about 15 per cent of the plasma volume

TABLE I

Data on patients to whom DFP^{32} was administered

Patient	Age	Sex	Clinical diagnosis	Body weight	Dose DFP^{32}	
	yrs.			*kg.*	*mg.*	*μc.*
A	68	M	Healed thrombosis	70	0.515	90
B	17	M	Duodenal ulcer	71	0.654	61
C	71	M	Asthma	66	1.37	107
D	49	M	Duodenal ulcer	75	0.411	40
E	66	F	Chronic myeloid leukemia, thrombocytosis	39	0.63	102
F	59	M	Polycythemia vera thrombocytosis	91	1.37	124
G	69	F	Apoplexy	85	1.09	70
H	57	F	8 days after cholecystectomy	80	1.2	150
I	49	M	8 days after nailing operation for fracture of the femur head	69	1.0	130
K	47	M	Thrombocytosis after splenectomy	69	1.17	145
L	63	F	Thrombocytopenic purpura due to gold in remission after ACTH therapy	47	1.1	140
M	51	M	Polycythemia vera splenomegaly low platelet count	70	0.92	120
N	77	M	Polycythemia vera splenomegaly low platelet count	71	0.8	110

is encountered. The platelet-rich plasma is centrifuged for 15 minutes applying a force of 220 G. Most of the leucocytes and erythrocytes (if present) are then collected on the bottom of the tube, the erythrocytes being clearly visible as a red layer. On the top of this the platelets are already partly sedimented. These are resuspended, every precaution being taken to prevent stirring of the bottom layer. The plasma is pipetted into another tube; it should never contain more than one erythrocyte or leucocyte per 5000 platelets. If too much contamination with other cells is present the separation has to be repeated. In order to isolate more blood platelets from the same blood sample the upper third of the compacted red cells and the portion of the plasma that has not been pipetted off during the first procedure are transferred to another centrifuge tube. By the same procedure of differential centrifugation as described above a second quantity of platelet-rich plasma can be prepared. The plasma thus collected is thoroughly mixed. A small sample is used for the determination of the number of platelets by counting in duplicate, a 3.8 per cent sodium citrate solution being used as diluting agent. At the the same time the number of erythrocytes and leucocytes still present is determined. The total amount of plasma obtained is measured and is centrifuged for 40 minutes applying a force of 2750 G. After the supernatant plasma has been discarded the resultant platelet mass is washed three times with saline. If more platelets have to be collected all cell masses left are resuspended in the plasma that has been freed of platelets and the whole procedure is repeated once more. In this way the platelet yield from the same sample can be increased by about 20 per cent. Control estimations of the radioactivity of the washings indicate that the washing procedure does not interfere with the platelet-bound radioactivity.

Measurement of the radioactivity. The platelets are hydrolyzed for 30 minutes at 100° C. in 3 ml. 30 per cent sodium hydroxide. The volume is made up to 10 ml. and the radioactivity of this final solution is measured as described before, using a Geiger Müller liquid counter. By relating the measurements to controls obtained by alkaline hydrolysis of labelled diisopropylfluorophospho-

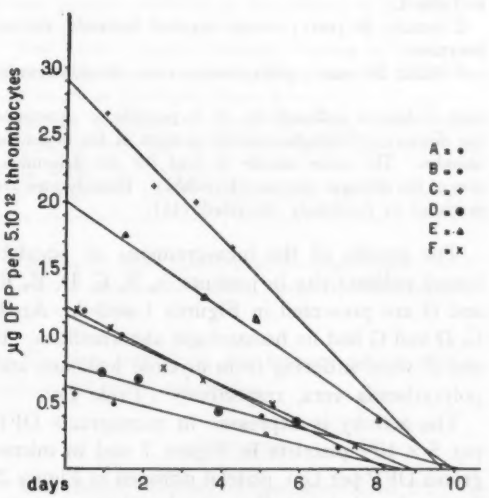


FIG. 1. PLATELET-BOUND RADIOACTIVITY AFTER INJECTION OF DFP^{32}

Activity expressed in μg. of DFP per 5×10^{12} platelets in the blood of subjects A, B, C, D, E, and F. Curves drawn by method of least squares.

A, B, C and D patients without hematologic abnormalities. For details about these patients the reader is referred to Table I.

E female; 66 years; chronic myeloid leukemia, thrombocytosis.

F male; 59 years; polycythemia vera, thrombocytosis.

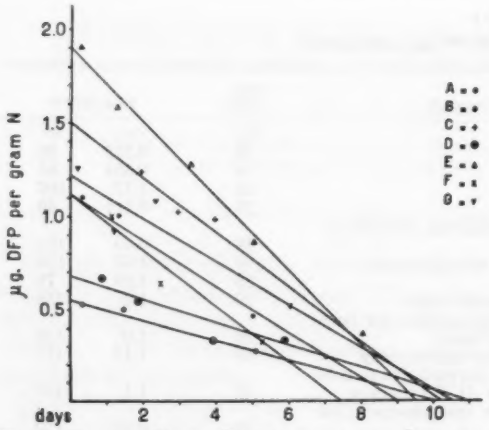


FIG. 2. PLATELET-BOUND RADIOACTIVITY AFTER INJECTION OF DFP^{32}

Activity expressed in µg. of DFP per Gm. of platelet nitrogen in the blood of subjects A, B, C, D, E, F, and G. Curves drawn by method of least squares.

A. B, C, D and G patients without hematologic abnormalities.

For details about these patients the reader is referred to Table I.

E female; 66 years; chronic myeloid leukemia, thrombocytosis.

F male; 59 years; polycythemia vera, thrombocytosis.

nate of known radioactivity, it is possible to determine the diisopropylfluorophosphonate content of the measured samples. The same sample is used for the determination of the nitrogen content (Kjeldahl). Hemolysates are prepared as previously described (15).

The results of the measurements of platelet-bound radioactivity in patients A, B, C, D, E, F, and G are presented in Figures 1 and 2. A, B, C, D and G had no hematologic abnormalities. E and F were suffering from myeloid leukemia and polycythemia vera, respectively (Table I).

The activity is expressed in micrograms DFP per 5×10^{12} platelets in Figure 1 and in micrograms DFP per Gm. platelet nitrogen in Figure 2. It was found that 1 Gm. platelet nitrogen corresponded with about 5×10^{12} platelets. The results do not differ significantly although the variation is somewhat higher where they are expressed per gram nitrogen. Probably this is due to technical difficulties in the determination of platelet nitrogen.

The linear relationship between time and platelet-bound radioactivity indicates that the destruction of platelets is attributable to a limited life span and not to random processes. The slope of the curves obtained suggests that the life span is about 9 days. This figure might possibly be somewhat exaggerated in some cases owing to contamination with radioactivity from other cells. Contamination reveals itself sometimes as a slight residual radioactivity after the ninth day. Since labelled thrombocytes disappear more rapidly from the circulation as a result of their shorter life span, the interference of contaminating red cells increases with time. However the error in the calculation as a result of contamination with other cells is no more than one day at most. The normal life span of the platelets can therefore be estimated as 8 to 9 days. Determination of the amount of DFP^{32} bound to leucocytes after isolation of these cells from the peripheral blood showed a high uptake 6 hours after injection with DFP^{32}. However, in leucocytes isolated one or more days after injection all, or nearly all, activity had disappeared. Because of this high uptake in the leucocytes correction for even a small contamination with leucocytes of a platelet sample may be necessary.

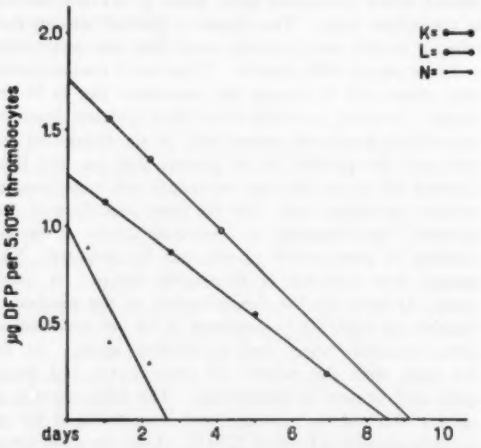


FIG. 3. PLATELET-BOUND RADIOACTIVITY AFTER THE INJECTION OF DFP^{32}

Activity expressed in µg. of DFP per 5×10^{12} platelets in the blood of subjects K, L, and N.

K male; 47 years; persistent thrombocytosis after splenectomy.

L female; 63 years; thrombocytopenic purpura due to gold, in remission after ACTH treatment.

N male; 77 years; polycythemia vera, splenomegaly, thrombopenia (platelet count about 90,000 per $mm.^3$ blood), some megakaryocytic hyperplasia in the bone marrow.

The values obtained in patient E (chronic myeloid leukemia) and patient F (polycythemia vera) suggest that in these two cases, both with a thrombocytosis and without splenomegaly, the survival time of the platelets did not differ from normal.

Owing to a technical error the results of platelet-bound radioactivity in patient G can only be given per Gm. of N.

Quite different were the results obtained in the two patients (M and N) with polycythemia vera with low platelet counts (about 100,000 per mm.^{3}). In both patients a splenomegaly was prominent and examination of sternal marrow showed an increased number of megakaryocytes with normal platelet formation. In patient M no platelet-bound radioactivity could be demonstrated one day after injection of DFP^{32}, though there was a normal uptake of DFP^{32} in the erythrocytes.

The results obtained in patient N are presented in Figure 3. On the third day after injection no more platelet-bound radioactivity could be detected. The data obtained in these two patients suggest a shortened life span of the platelets.

Figure 3 also includes data obtained on patients K and L; both these cases were of special interest. Patient L had suffered from a severe thrombocytopenic purpura due to gold treatment. She was in complete remission after ACTH therapy. The peripheral platelet counts during the time of the experiment were maintained at about 250,000 per mm.^{3} The data obtained indicate that no abnormal breakdown of the platelets was taking place.

Patient K had undergone a splenectomy for an enlarged spleen two and a half years before admission. At operation he was found to have a very large infarcted spleen. Histological examination showed an increase in fibrous tissue. The splenectomy was followed by a persistant thrombocytosis associated with thrombotic and hemorrhagic manifestations. Sternal marrow examination revealed a cellular marrow with numerous megakaryocytes showing abundant platelet formation. The data obtained in this splenectomized patient suggest that the removal of the formed platelets from the blood was proceeding at a normal rate.

Results of the measurements of platelet bound radioactivity in the two postoperative patients I and H are presented in Figure 4.

The interpretation of these curves with regard to life span is difficult for reasons which will be elaborated in the discussion.

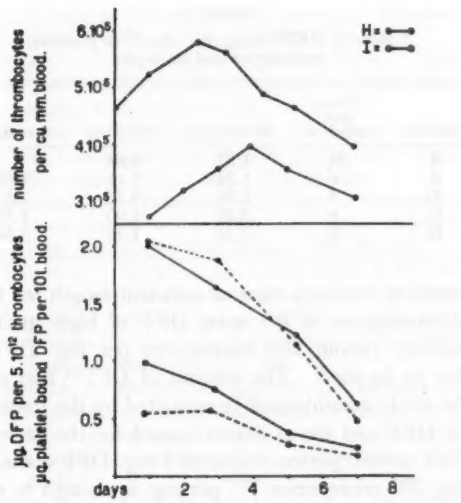


FIG. 4. THE UPPER GRAPH PRESENTS THE VALUES FOR THE PLATELET COUNTS IN THE BLOOD OF SUBJECTS H AND I

Platelet counts were performed in duplicate using calibrated pipettes according to the method of Feissly and Lüdin (19).

THE LOWER GRAPH PRESENTS VALUES OF PLATELET-BOUND RADIOACTIVITY AFTER INJECTION OF DFP^{32} IN THE BLOOD OF SUBJECTS H AND I

Activity expressed in µg. of DFP per 5×10^{12} platelets (solid lines) and in µg. of platelet-bound DFP per 10 L. blood (broken lines).

H female; 57 years; cholelithiasis; DFP^{32} injected 8 days after cholecystectomy; postoperative course uneventful.

I male; 49 years, fracture of the femur head; DFP^{32} injected 8 days after nailing operation. Postoperative course uneventful.

DISCUSSION

The results obtained demonstrate that DFP is bound to platelets. Expressed per Gm. of nitrogen the amount of platelet-bound radioactivity is somewhat lower than the amout of radioactivity bound to the erythrocytes. In Table II comparative figures of the uptake of DFP^{32} in erythrocytes, platelets and leucocytes 6 and 24 hours after injection are given. Measurement of platelet-bound radioactivity will be most reliable during the first days after injection. In order to obtain an uptake of radioactivity high enough to permit meas-

TABLE II

Uptake of DFP^{32} in μg. per Gm. N in platelets, erythrocytes and leucocytes

Patients	Hours after injection	Hemolysate	Platelets	Leucocytes
A	24	1.52	0.68	
B	6	1.34	1.11	1.08
D	6	1.06	0.53	1.32
E	6	2.20	1.90	4.50
G	6	2.36	1.25	3.50

urement during a time of sufficient length for the determination of life span, DFP of high specific activity (about 200 microcuries per mg. DFP) has to be used. The amount of DFP^{32} that can be safely administered is restricted by the toxicity of DFP and the radiation hazard for the patient. In a normal person a dose of 1 mg. DFP containing 200 microcuries P^{32} per mg. is enough to obtain platelet samples with a radioactivity of about ten times the background during several days after injection. The dosages, used by us, are entirely safe in both respects.

There is no reason to assume that the resorption of DFP from the muscular depots will last much longer than a few hours after the injection. Firstly, the maximum radioactivity in the total blood is reached in one and a half hours after the injection. Secondly, the excretion of DFP^{32} during the first 24 hours after the injection amounts to about 30 per cent of the administered dose; in the same time about 20 per cent has been bound by the erythrocytes. This means that urinary excretion and binding to the erythrocytes account for at least 50 per cent of the injected dose within 24 hours. The results do not suggest that there has been an important delivery of marked cells from the bone marrow. Large variations in the radioactivity and in the amount of DFP administered prevailed throughout our experiments, without affecting the figures obtained for the life span. This suggests that radiation or toxic effects do not seriously interfere with the life span of the platelets under our conditions.

The method has proved useful in the determination of the life span of platelets in hematologically normal subjects. It was found to amount to 8 to 9 days. The same normal values were found in several patients suffering from various hematologic disorders with normal and high platelet counts. It is obvious that the method, involving platelet labelling *in vivo*, will be most satisfactory at normal or increased platelet values. In thrombopenic states the low availability of platelets that can be labelled might limit the applicability. In two cases of polycythemia vera with thrombopenia (patients M and N), significantly shortened life spans were observed. In patient M strong evidence also existed that the life span of the erythrocytes was markedly reduced; there was a persistent reticulocytosis, a high hemolytic index, and a life span of the erythrocytes determined with DFP^{32} of about seventy days. In these two patients with thrombocytopenia, determination of the life span of the thrombocytes was made easier because the thrombocytopenia was not so severe and the blood cell volume was high making it possible to take a large blood sample for the isolation of the platelets.

The possibility that alteration of platelet material involving a modified reaction with DFP might lead to incorrect interpretation of the life span values should be considered.

It has been suggested (18) that in cases of polycythemia vera the finding of a normal platelet count might be due to increased production of platelets compensated by a shortened survival of these elements. The present method could be useful in elucidating this condition.

Results suggestive of a normal survival were obtained in two postoperative patients (H and I). However the interpretation of the data obtained (Figure 4) is complicated. First, the total thrombocyte count is not maintained at a stable level owing to the postoperative rise in platelet production. To obtain curves which would still faithfully express the rate of platelet breakdown it has been necessary to plot the radioactivity per volume of blood instead of per number of platelets (Figure 4). The same factor results in an uneven distribution of platelets among the various age groups with a resultant unequal rate of elimination. This would change the pattern in such a way that the later part of the curve would tend to have a steeper slope than the initial portion. The present curves might possibly be interpreted as showing the pattern to be expected on the basis of a normal survival time. However, additional data are required to justify the conclusion that the platelets enjoy a normal survival under these conditions. Any attempt to interpret postoperative platelet curves must be based on the knowledge of the life span of the platelets under these circumstances.

SUMMARY

The uptake of DFP^{32} by the platelets was determined in 5 subjects without hematologic abnormalities, in 6 patients with hematologic abnormalities and in two postoperative patients. Results of the determination of platelet-bound radioactivity during several days after the injection of DFP^{32} are presented. A linear relationship between time and platelet-bound radioactivity was found indicating a measurable life span of these elements under normal circumstances. The slope of the curves obtained suggested that the life span amounts to about 8 to 9 days. In two patients suffering from chronic myeloid leukemia and polycythemia vera, respectively, both with thrombocytosis a normal survival time of the platelets was found. The data obtained on two patients with polycythemia vera with low platelet counts strongly suggested a considerable decrease of the life span of the platelets. Estimations carried out on two patients during the second week following an operation, though difficult to interpret owing to a fluctuating platelet count, are not inconsistent with a normal life span of the platelets under these conditions.

ACKNOWLEDGMENT

Our thanks are due to Professor Dr. A. Querido, for making his facilities available.

REFERENCES

1. Leeksma, C. H. W., and Cohen, J. A., Determination of the life of human blood platelets using labelled diisopropylfluorophosphonate. Nature, 1955, **175**, 552.
2. Dillard, G. H. L., Brecher, G., and Cronkite, E. P., Separation, concentration and transfusion of platelets. Proc. Soc. Exper. Biol. & Med., 1951, **78**, 796.
3. Hirsch, E. O., and Gardner, F. H., The transfusion of human blood platelets with a note on the transfusion of granulocytes. J. Lab. & Clin. Med., 1952, **39**, 556.
4. Gardner, F. H., Howell, D., and Hirsch, E. O., Platelet transfusions utilizing plastic equipment. J. Lab. & Clin. Med., 1954, **43**, 196.
5. Hirsch, E. O., Gardner, F. H., and Thomas, E. D., Isolation and concentration of human blood platelets: Their properties *in vitro* and *in vivo*. J. Clin. Invest., 1952, **31**, 638.
6. Stefanini, M., Chatterjea, J. B., Dameshek, W., Zannos, L., and Santiago, E. P., Studies on platelets II. The effect of transfusion of platelet-rich polycythemic blood on the platelets and hemostatic function in "idiopathic" and "secondary" thrombocytopenic purpura. Blood, 1952, **7**, 53.
7. Odell, T. T., Jr., Gamble, F. N., and Furth, J., Life span of naturally labelled platelets of rats. Federation Proc., 1953, **12**, 398.
8. Morgan, M. C., Keating, R. P., and Reisner, E. H., Jr., Labeling of rabbit platelets with iodine^{131}. Proc. Soc. Exper. Biol. & Med., 1954, **85**, 420.
9. Mueller, J. F., Pathologic physiology of mammalian blood platelet utilizing P^{32}tagged rabbit platelets. Proc. Soc. Exper. Biol. & Med., 1953, **83**, 557.
10. Robertson, J. S., Milne, W. L., and Cohn, S. H., Labelling and tracing rat blood platelets with Chromium-51 *in* Radio Isotope Conference, 1954, Proceedings of the Second Conference, New York, Academic Press, Vol. 1, p. 205.
11. Julliard, J., Maupin, B., Loverdo, A., Bernard, J., Colvez, P., et Leconte, M., Premiers essais de transfusion a l'homme de leucocytes et de plaquettes marqués au radiophosphore. Presse méd., 1952, **60**, 518.
12. Desai, R. G., Small, W., and Mednicoff, I., Studies on the survival and metabolic activity of platelets in humans, utilizing radio-active phosphorus. J. Clin. Invest., 1955, **34**, 930.
13. Odell, T. T., Jr., Tausche, F. G., and Furth, J., Platelet life span as measured by transfusion of isotopically labelled platelets into rats. Acta Hemat., 1955, **13**, 45.
14. Morgan, M. C., Keating, R. P., and Reisner, E. H., Jr., Survival of radio-chromate-labeled platelets in rabbits. J. Lab. & Clin. Med., 1955, **46**, 521.
15. Cohen, J. A., and Warringa, M. G. P. J., The fate of P^{32} labelled diisopropylfluorophosphonate in the human body and its use as a labelling agent in the study of the turnover of blood plasma and red cells. J. Clin. Invest., 1954, **33**, 459.
16. Alexander, B., Some biochemical, physiological and pathological aspects of the coagulation mechanism *in* Blood Cells and Plasma Proteins, Their State in Nature, Tullis, J. L., Ed., New York, Academic Press, 1953, p. 78.
17. Oosterbaan, R. A., van Rotterdam, J., and Kunst, P., Synthesis of diisopropylphosphorofluoridate labelled with ^{32}P ($DF^{32}P$). Report Med. Biol. Lab. Nat. Def. Res. Council T.N.O. No. 31 (November 1955).[1]
18. Black, M. M., Preston, J. A., and Speer, F. D., Histochemical and quantitative studies of the *in vitro* dehydrogenase activity of spleen in hypersplenism. Blood, 1955, **10**, 145.
19. Feissly, R., et Lüdin, H., Microscopie par contrastes de phases (III. Applications à l'hématologie). Rev. d'hémat., 1949, **4**, 481.

[1] The method described in this report will appear shortly in more detail in the generally available literature. In the meantime a limited number of copies will be made available to workers in the field on application to Dr. J. A. Cohen.

THE RELATIONSHIP OF CARDIOVASCULAR AND RENAL HEMODYNAMIC FUNCTION TO SODIUM EXCRETION IN PATIENTS WITH SEVERE HEART DISEASE BUT WITHOUT EDEMA [1, 2]

By WILLIAM HOLLANDER and WALTER E. JUDSON [3]

(From the Robert Dawson Evans Memorial Department of Clinical Research and Preventive Medicine, Massachusetts Memorial Hospitals and the Department of Medicine, Boston University School of Medicine, Boston, Mass.)

(Submitted for publication March 19, 1956; accepted May 17, 1956)

Patients in congestive heart failure with peripheral edema are known to have an impairment of sodium excretion (1), which may persist for some time after they recover from clinical "heart failure" (2). However, little information is available concerning the sodium excretion of patients with severe heart disease and dyspnea but without a history of peripheral edema. Therefore, the present study was undertaken to investigate sodium excretion in such non-edematous cardiac patients during a moderately high sodium intake. When it was found that this group had no obvious disturbance in the renal excretion of sodium, measurements were made of their cardiovascular and renal hemodynamic functions both at rest and during exercise. These measurements were compared with similar determinations in a group of patients with severe heart disease but with peripheral edema in order to determine whether circulatory differences existed which might account for the observed differences in renal excretion of sodium.

PATIENTS, METHODS AND COLLECTION OF DATA

The first group of cardiac patients had dyspnea on exertion but no history or finding of peripheral edema although some had previously received an occasional diuretic. Most of the subjects had severe valvular heart disease and evidences of pulmonary congestion in spite of the fact that they were on maintenance doses of digitalis. None had received a diuretic for at least three weeks prior to the study. Simultaneous cardiovascular and renal hemodynamic measurements were made at rest and during exercise by methods identical to those previously described (3). Exercise was performed in the supine position by alternate straight leg-raising for six to nine minutes.

Within one week after these measurements were made, each patient was placed on a diet unrestricted in salt for three to five days. Following this, he was placed on a diet containing 1 gram of sodium chloride, and, in addition, was given oral tablets containing 13 grams of sodium chloride a day. This regimen was continued for 7 to 14 days during which time the patient was ambulatory but was observed for changes in symptoms or weight. Twenty-four-hour collections of urine were analyzed for sodium and chloride content at intervals of two to three days. Within a week after the completion of this study, ten of the twelve patients were studied during and after an infusion of 300 ml. of 5 per cent sodium chloride given at a rate of 10 to 12 ml. per minute. Sodium and chloride excretions were measured over the ensuing 24-hour period by taking urine collections at the end of the infusion, one hour later, and then at intervals of about six hours. Control observations were obtained by making similar studies on nine normal individuals. Urinary sodium was determined by the internal standard (lithium) flame photometer. Urinary chlorides were analyzed by the method of Wilson and Ball (4).

The second group of 10 cardiac patients were in congestive heart failure with peripheral edema. They had considerable "right sided failure" as indicated by the degree of venous distention and hepatomegaly. Most of these patients received 1 gram of salt daily in the diet, a maintenance dose of digitalis, and frequent injections of a mercurial diuretic. Cardiovascular and renal hemodynamic functions in this group were studied in the same way as the first group.

RESULTS

Sodium excretion

The data for sodium chloride balance in patients without edema of group I are shown in Table I.

The "non-edematous" patients (who had never had a history of edema) failed to gain weight or develop any change in their signs or symptoms during the entire seven to fourteen days during which they were maintained on a diet containing 14

[1] Presented at the annual meeting of the American Heart Association, Chicago, Illinois, April 4, 1954.

[2] This investigation was supported in part by a grant from the National Heart Institute of the National Institutes of Health, U.S.P.H.S.

[3] Present address: Department of Medicine, Indiana University Medical Center, Indianapolis, Ind.

TABLE I

Sodium chloride excretion in a group of cardiac patients without peripheral edema *

Patient Age	Sex	Clinical classification	Days on 14 gram (approx.) NaCl diet	Change† in weight (*pounds*)	Urinary Na conc. (*mEq./L.*) Average	Urinary NaCl excretion in 24 hours (*gm.*) Average	Changes in signs and symptoms
C. S.		R.H.D. M.S.	13	+ .50	136	13.2	None
37	M	N.S.R. III D					
A. F.		R.H.D. M.I.	14	− .75	145	12.8	None
32	F	A.F. IV E					
V. C.		R.H.D. M.I.	10	+1.00	94	12.6	None
32	F	A.F. III D					
S. A.		R.H.D. M.S.	12	+ .25	126	12.9	None
41	F	N.S.R. III D					
J. D.		R.H.D. M.I.	14	0	155	12.7	None
39	M	A.F. IV E					
C. J.		A.S.H.D. R.H.D.	7	−1.25	137	13.4	None
49	M	M.S. A.F. III D					
E. M.		R.H.D. N.S.R.	10	+1.00	157	13.5	None
48	F	III D					
A. H.		Chron. Pulm. Emphysema	7	− .5	95	12.5	None
47	M	Cor pulmonale N.S.R. IV E					
D. C.		H.C.V.D. A.F.	10	+1.25	105	14.0	None
48	M	III C					
A. C.		R.H.D. M.S.	7	−1.5	132	12.0	None
40	M	N.S.R. II C					
E. T.		R.H.D. M.S.	10	+ .5	140	12.5	None
22	F	A.I. N.S.R. III C					
M. M.		Sarcoid Pulm. Emph. & Fibrosis	10	−2.00	117	14.4	None
22	M	Cor pulmonale II C					

* Approx.—Approximately.
R.H.D.—Rheumatic Heart Disease.
A.S.H.D.—Arteriosclerotic Heart Disease.
H.C.V.D.—Hypertensive Cardiovascular Disease.
A.F.—Auricular Fibrillation.
N.S.R.—Normal Sinus Rhythm.
M.S.—Mitral Stenosis.
M.I.—Mitral Insufficiency.
A.I.—Aortic Insufficiency.
Chronic Pulm. Emph.—Chronic Pulmonary Emphysema.
Sarcoid Pulm. Emph. and Fibrosis—Sarcoid Pulmonary Emphysema and Fibrosis.

† The difference between the weight obtained on the day preceding the 14-gram NaCl diet and that on the last day of the diet.

TABLE II

Individual cardiovascular and renal data

Patient Age	Sex	Clinical classification	Cardiac index *L./min./m²* R	E	Oxygen consumption *ml./min./m²* R	E	A-V oxygen difference *Vol. %* R	E	Arterial oxygen saturation % R	E
							Group A. Patients with Cardiac			
C. S. 37	M	R.H.D. M.S. N.S.R. III D	2.67	3.19	179	427	6.73	13.36	93	90
A. F. 32	F	R.H.D. M.I. A.F. IV E	1.46	1.82	139	222	9.51	12.18	96	100
V. C. 32	F	R.H.D. M.I. A.F. III D	2.19	3.65	145	371	6.59	10.20	95	94
S. A. 41	F	R.H.D. M.S. N.S.R. III D	3.12	3.58	140	355	4.51	9.94	94	96
J. Di. 39	M	R.H.D. M.I. A.F. IV E	1.60	3.21	139	389	8.70	12.12	95	98
C. J. 49	M	A.S.H.D. R.H.D. M.S. A.F. III D	1.99	3.09	143	358	7.16	11.60	96	100
E. M.* 48	F	R.H.D. N.S.R. III D	2.24		127		5.66	9.71	89	86
A. H. 47	M	Chron. Pulm. Emphysema Cor pulmonale N.S.R. IV E	2.82	4.45	116	196	4.10	4.40	44	32
D. C. 48	M	H.C.V.D. A.F. III C	4.30	6.73	211	606	4.90	9.00	93	93
A. C. 40	M	R.H.D. M.S. N.S.R. II C	3.27	4.79	150	470	4.57	9.83	99	94
E. T.* 22	F	R.H.D. M.S. A.I. N.S.R. III C	3.28	4.58	146	377	4.42	8.24	94	92

* Developed pulmonary edema during exercise.

R.H.D.—Rheumatic Heart Disease.
A.S.H.D.—Arteriosclerotic Heart Disease.
H.C.V.D.—Hypertensive Cardiovascular Disease.
A.F.—Auricular Fibrillation.
N.S.R.—Normal Sinus Rhythm.
M.S.—Mitral Stenosis.
M.I.—Mitral Insufficiency.
Thyroid H.D.—Thyroid Heart Disease.

TABLE II—*Continued*

Pressures (*mm. Hg*)															
Pulmonary artery					Mean pulmonary capillary		Right ventricular end diastolic		Systemic arterial				Pulse rate *Beats/min.*		C_{PAH} *ml./min./1.73 m²*		C_{IN} *ml./min./1.73 m²*	
S	D	M	S	D M	R	E	R	E	S	D M	S	D M	R	E	R	E	R	E
R			E						R		E							
Insufficiency without Peripheral Edema																		
102	47	65	138	61/99	30	41	10	18	130	80/100	170	100/135	85	136	256	220	95	82
43	25	30	62	38/48	26	35	7	10	110	62/80	135	75/90	90	129	357	268		
40			65				12	30	110	65/85	140	80/100	82	160	477	207		
72	46	57	130	75/100	32	45	5	10	95	60/80	118	75/95	84	128	448		97	
50	32	36	80	38/50			14	18	110	80/90	125	80/100	88	136	206	106	94	44
50	20	30	60	25/38			12	15	160	90/105	160	90/105	87	126	279	332	104	123
76	38	55	110	52/75	34	55	5	10	170	90/120	265	150/207	77	104	267	94	98	42
94	60	72	105	65/78	18	22	14	24	160	80/105	165	90/110	105	108	313	200	138	117
59	31	40	80	42/55	27		13	19	204	84/135	260	114/180	84	120	250	184	76	59
45	35	40	90	60/68	30	48	5	20	138	80/100	140	80/100	78	138	268	140	76	54
45	20	33	82	46/65	20	40	2	5	105	60/80	125	72/88	62	110	486	324	127	36

A.I.—Aortic Insufficiency.
C.P.—Cor pulmonale.
A.S.—Aortic Stenosis.
P.E.—Pulmonary Emphysema.

Chron. Pulm. Emphysema—Chronic Pulmonary Emphysema.
Sarcoid Pulm. Emphysema and Fibrosis—Sarcoid Pulmonary Emphysema and Fibrosis.

TABLE II—*Continued*

Patient Age	Sex	Clinical classification	Cardiac index *L./min./m²* R	E	Oxygen consumption *ml./min./m²* R	E	A-V oxygen difference *Vol. %* R	E	Arterial oxygen saturation *%* R	E
							Group A. Patients with Cardiac Insufficiency			
M. M. 22	M	Sarcoid Pulm. Emph. and fibrosis C.P. II C	3.05	5.33	154	368	5.06	6.90	87	87
							Group B. Patients with Cardiac			
F. A. 61	F	A.S.H.D. A.F. III D	2.10	2.46	122	266	5.78	10.79	97	98
M. R. 39	M	H.C.V.D. N.S.R. IV E	1.46		167		11.40		95	
R. A. 54	M	H.C.V.D. N.S.R. IV D	2.40	3.50	176	395	7.32	11.13	94	92
L. G. 36	F	R.H.D. A.S. N.S.R. III C	1.97	2.97	163	312	8.30	10.51	96	
C. M. 42	F	Thyroid H.D. A.F. III C	4.62		205		4.44		93	
C. J. 42	F	R.H.D. M.S. A.F. IV E	2.10	2.27	216	315	10.13	13.89	88	90
J. C. 45	M	R.H.D. M.S. A.F. III C	2.74	4.06	158	391	5.76	9.65	93	88
W .T. 40	M	R.H.D. A.S., M.I., and M.S. A.F. III D	2.38		161		6.76		95	
W. H. 56	M	P.E. C.P. N.S.R. IV E	3.49	3.84	154	283	4.42	7.37	75	67
W. D. 66	M	Silicosis C.P. N.S.R. III D	3.65	3.50	137	215	3.75	6.12	52	51

TABLE II—*Continued*

Pressures (*mm. Hg*)																				
Pulmonary artery						Mean pulmonary capillary		Right ventricular end diastolic		Systemic arterial						Pulse rate *Beats/min.*		C_{PAH} *ml./min./1.73 m²*		C_{IN} *ml./min./1.73 m²*	
S	D	M	S	D	M					S	D	M	S	D	M						
R			E			R	E	R	E	R			E			R	E	R	E	R	E
without Peripheral Edema—*Continued*																				
53/30/38			70/40/48					8	12	120/90/102			148/100/120			92	116	266	180	84	77
Insufficiency and Peripheral Edema																				
60/40/45			85/57/62			27		18	22	118/66/84			128/78/90			92	116	143	130	56	46
72/53/60								30		220/160/180						106		171		113	
85/47/60			125/75/95			40	55	22	45	195/90/120			280/140/180			88	104	240	162	92	62
98/48/65								27		95/65/75						86		180		82	
65/34/45								20		180/70/115						110		462		103	
140/80/100			145/90/120			30	40	23	28	180/120/145			200/120/160			90	112	221	177	98	80
70/38/49			95/50/70			33	45	20	25	140/70/100			180/80/110			74	108	394	299	117	89
68/36/50			120/62/78					19	38	140/80/100			150/90/110			52	60	316		104	
60/44/50			75/50/60			18	25	22	25	130/80/90			120/60/85			94	110	212	187	91	87
81/42/53			110/58/68			18	18	23	30	150/75/100			210/90/130			92	112	212	106	91	58

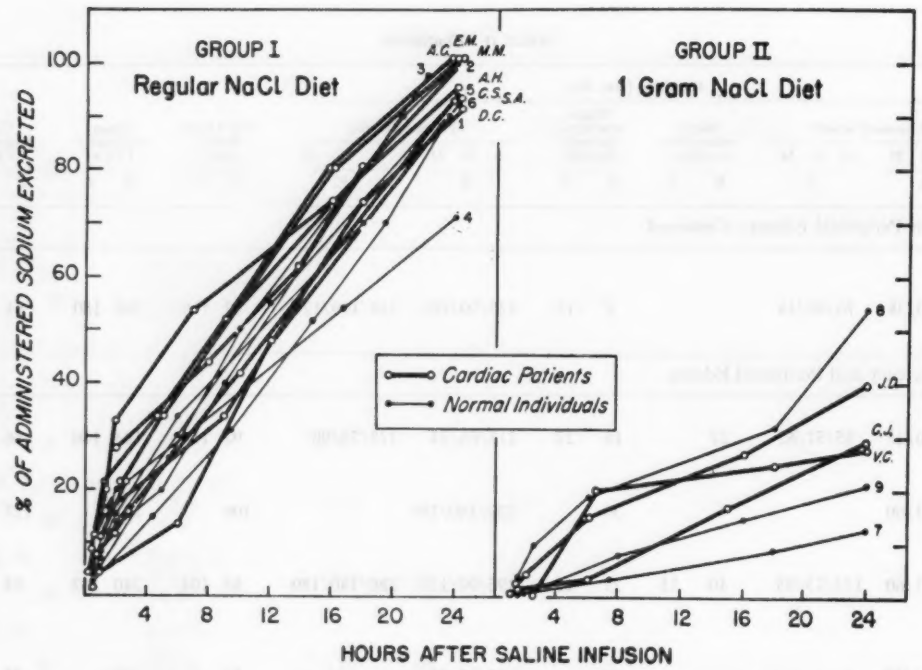


FIG. 1. URINARY EXCRETION OF SODIUM FOLLOWING THE INFUSION OF 300 ML. OF 5 PER CENT SALINE

grams of sodium chloride daily. The 24-hour urinary sodium excretion showed good concentration and a high recovery of the ingested salt, averaging 93 per cent for the group. All except patients A. F., S. A., C. J., and A. H. were followed carefully on an unrestricted salt diet for at least three months following this study and they continued to be edema free and showed no change in cardiac symptoms or signs.

Figure 1 shows graphically the excretion of sodium in this group following the infusion of 300 ml. of 5 per cent sodium chloride. Seven of the ten patients, like the normal controls, showed no delay in the excretion of sodium including even patient E. M. who developed cardiac asthma during the infusion. The three remaining patients, one of whom (V. C.) also developed acute pulmonary edema during the saline infusion, had previously been on a diet restricted in sodium and all three had a delay in excretion of sodium. This, however was comparable to that observed in three normal individuals who had likewise been on a limited sodium intake.

Hemodynamic functions

The cardiovascular and renal hemodynamic functions in this group of non-edematous cardiac patients are given in Table II for comparison with those in the edematous cardiac patients. A statistical analysis of the differences between these two groups is given in Table III.

Cardiac index and oxygen consumption. At rest the cardiac index was abnormally low and not significantly different in the two cardiac groups. During exercise the group without edema had a higher mean cardiac index (by 0.81 L. per min. per m^2) than the group with edema but this difference was not statistically significant. At rest and during exercise, the oxygen consumption and A-V O_2 difference likewise were not significantly different in the two groups. In response to exercise both groups had significant rises in cardiac index. The increase in this function, however, was significantly greater in the group without edema than the group with edema. This difference between the groups in response to exercise was not associated with a significantly different arteriovenous oxygen

TABLE III

Mean data and statistical analysis

	Cardiac index *L./min./M²*				Oxygen consumption *ml./min./M²*				A-V oxygen difference *Vol. %*			
	Rest	Exercise	Response to exercise	P	Rest	Exercise	Response to exercise	P	Rest	Exercise	Response to exercise	P
Group A	2.67±0.24	4.04±0.40	+1.33±0.21	<0.01	149± 7	376±33	+225±27	<0.01	5.99±0.51	9.79±0.72	+3.80±0.48	<0.01
Group B	2.69±0.30	3.23±0.25	+0.59±0.21	0.03	166± 9	311±25	+150±22	<0.01	6.81±0.80	9.92±0.97	+3.43±0.37	<0.01
Difference	−0.02±0.38	+0.81±0.47	+0.74±0.29		−17±11	+65±41	+ 75±35		−0.82±0.94	−0.13±1.21	+0.37±0.61	
Significance of Difference (P)	>0.9	0.1	0.02		0.2	0.1	0.05		0.4	>0.9	0.6	

	Arterial oxygen saturation %				Pressures (*mm. Hg*) Pulmonary arterial: Systolic				Diastolic			
	Rest	Exercise	Response to exercise	P	Rest	Exercise	Response to exercise	P	Rest	Exercise	Response to exercise	P
Group A	90±4	88±5	−1±1	0.4	61± 6	89± 8	+28±4	<0.01	35±4	49±4	+14±3	<0.01
Group B	88±4	81±7	−2±2	0.2	80± 8	108± 9	+27±6	<0.01	46±4	63±6	+16±3	<0.01
Difference	+2±6	+7±9	+1±2		−19±10	−19±12	+1±7		−11±6	−14±7	−2±4	
Significance of Difference (P)	0.8	0.4	0.6		0.07	0.1	0.9		0.06	0.07	0.6	

	Pressures (*mm. Hg*) Pulmonary arterial (mean)				Right ventricular end diastolic				Systemic arterial (systolic)			
	Rest	Exercise	Response to exercise	P	Rest	Exercise	Response to exercise	P	Rest	Exercise	Response to exercise	P
Group A	45±4	66± 6	−21±4	<0.01	9±1	16±2	+7±1	<0.01	134± 9	162±14	+28± 8	<0.01
Group B	58±5	79± 8	+21±3	<0.01	22±1	30±3	+9±3	0.02	155±12	181±21	+31±12	0.05
Difference	−13±7	−13±10	−0.1±5		−13±2	−14±4	−2±3		−21±15	−19±25	−3±15	
Significance of Difference (P)	0.08	0.2	>0.9		<0.01	<0.01	0.5		0.2	0.5	0.9	

	Pressures (*mm. Hg*) Systemic arterial (diastolic)				Systemic arterial (mean)				Pulse rate *Beats/min.*			
	Rest	Exercise	Response to exercise	P	Rest	Exercise	Response to exercise	P	Rest	Exercise	Response to exercise	P
Group A	77± 3	92± 6	+15±5	<0.01	99± 5	119±11	+21± 7	0.02	84±3	126±4	+41±5	<0.01
Group B	88±10	94±10	+11±8	0.2	111±10	123±13	+18± 8	0.07	88±5	109±4	+26±5	<0.01
Difference	−11±10	−2±12	+4±9		−12±11	−4±18	+3±11		−4±6	+17±6	+15±7	
Significance of Difference (P)	0.3	0.9	0.6		0.3	0.8	0.8		0.5	0.02	0.06	

	Clearances *vol./min./1.73 m²* PAH				Inulin			
	Rest	Exercise	Response to exercise	P	Rest	Exercise	Response to exercise	P
Group A	323±28	205±24	−106±25	<0.01	99±6	70±11	−29±11	0.03
Group B	255±33	177±27	−60±16	0.01	95±5	70± 7	−20± 5	<0.01
Difference	+68±43	+28±36	−46±29		+4±8	+0.1±13	−8±12	
Significance of Difference (P)	0.1	0.5	0.1		0.6	>0.9	0.5	

difference, but was associated with a significantly larger increase in oxygen consumption in the edema free than in the edematous group. Two cardiac patients without edema (D. C. and A. C.) who clinically had much less cardiac insufficiency than the rest of the patients in the study exercised so vigorously that they had by far the largest increases in oxygen consumption. If these two patients are excluded from the statistical analysis, the differences in both cardiac index and oxygen consumption between the groups in response to exercise lack statistical significance, the P values being .07 and .2, respectively. The arterial oxygen saturation at rest, during exercise and the changes in response to exercise did not differ significantly between the two groups. Although this measurement was reduced at rest, it did not change significantly with exercise.

Pulmonary arterial pressure. At rest and during exercise, the average systolic, diastolic and mean pulmonary arterial pressures were elevated in both groups, but were 11 to 19 mm. Hg lower in the group without edema than in the group with edema. The differences between the groups, however, lacked statistical significance at the 5 per cent level. The significant increases in pulmonary arterial pressures which occurred in both groups in response to exercise, were not significantly different from each other.

Right ventricular end-diastolic pressure. In the cardiac group without edema the right ventricular end-diastolic pressure at rest was slightly elevated whereas in the edematous group it was strikingly elevated. The difference was highly significant statistically. During exercise likewise the right ventricular end-diastolic pressure was significantly higher in the group with edema than the group without edema. In response to exercise both groups had significant increases in the right ventricular end-diastolic pressure which were not significantly different from each other.

Systemic arterial pressure. The average systolic, diastolic and mean arterial pressures at rest, during exercise and the changes in response to exercise were not significantly different between the groups. The increases in all of the components of the systemic arterial pressure were significant only in the non-edematous cardiac group.

Pulse rate. Statistical analysis showed that the pulse rates, which were not significantly different at rest, were significantly higher during exercise in the non-edematous than in the edematous group. The rise in pulse rate in response to exercise, however, just lacked being significantly different in the two groups.

Renal clearance measurements

PAH clearance. In both cardiac groups the renal plasma flow was reduced at rest. In response to exercise both groups had further decreases in this function which were statistically significant. The average PAH clearances at rest and during exercise were respectively 68 ml. per min. per 1.73 m^2 and 28 ml. per min. per 1.73 m^2 higher in the group without edema than in the group with edema. However, these differences between the groups (both at rest and during exercise) lacked statistical significance.

Inulin clearance. In both cardiac groups the glomerular filtration rate, which was reduced at rest, was significantly further depressed during exercise. The inulin clearance at rest, during exercise, and the change in response to exercise were not significantly different between the groups.

DISCUSSION

Cardiac patients with signs and symptoms of pulmonary congestion but with little or no evidence of "right-sided heart failure" do not necessarily develop salt and fluid retention when placed on a moderately high sodium intake. A majority of such patients, most of whom had valvular heart disease, were also found to have no obvious defect in the excretion of sodium when loaded acutely with hypertonic saline. Past studies, however, indicate that cardiac patients with previous "right sided failure" may have a persistent disturbance in sodium excretion even after "compensation" (2, 5). Since in the present study sodium excretion was determined only in cardiac patients without a history of peripheral edema, the previous findings do not necessarily conflict with the present results.

A number of the cardiac patients who tolerated a moderately high salt diet without developing edema had many hemodynamic as well as clinical findings considered to be indicative of severe cardiac insufficiency. In addition to pulmonary hypertension, their circulatory abnormalities included a reduction in cardiac output and a de-

crease in renal plasma flow and glomerular filtration rate. However, in none was the right ventricular end-diastolic pressure elevated above 14 mm. Hg. This latter hemodynamic function was the most consistent one which differentiated the cardiac group who had no evidence of salt and water retention from the group with such retention as indicated by the clinical finding of peripheral edema. In every one of the "salt-retaining group" right ventricular end-diastolic pressure was above 15 mm. Hg. Neither at rest nor during exercise were the cardiac output, pulmonary artery pressure, systemic arterial pressure, renal plasma flow and glomerular filtration rate consistently or significantly different in the two groups.

The present findings do not lend support to the "forward failure" concept as the predominant cause of edema formation in congestive heart failure. Rather they are more consistent with the "backward failure" concept in that they suggest that a critical elevation of the right ventricular end-diastolic pressure and peripheral venous pressure is the more important determining factor. However, it is obviously possible that reductions in cardiac output and glomerular filtration rate may also operate to increase the renal retention of sodium and water. Studies in experimentally induced heart failure also indicate that retention of salt and water occurs primarily as a result of the elevation of the venous pressure (6, 7).

Although venous hypertension may signal the danger of edema formation in congestive heart failure, edema may not form without an ample dietary intake of sodium chloride. A number of cardiac patients, while having a marked and persistent elevation of the venous pressure, have been observed to be edema free during a restricted intake of sodium. However, on increasing the sodium intake, these patients have developed salt retention and peripheral edema. The studies of Relman and Schwartz (8) may have a bearing on these observations in that they suggest that the action of salt retaining hormones, which appear to be increased in congestive heart failure (9, 10) are affected by the sodium content of diet.

SUMMARY

Cardiac patients who had many clinical and hemodynamic findings of congestive heart failure but no history of peripheral edema were found to tolerate a moderately high sodium intake without developing edema or salt retention. Many in the group likewise showed no impairment in sodium excretion when infused with 5 per cent saline. The most consistent hemodynamic measurement that differentiated this group from a similar cardiac group with peripheral edema was the finding of a lower right ventricular end-diastolic pressure. This finding supports the concept that salt retention in congestive heart failure may be initiated primarily by a critical rise in the venous pressure rather than by a decrease in cardiac output or glomerular filtration rate.

REFERENCES

1. Futcher, P. H., and Schroeder, H. A., Studies on congestive heart failure; II. Impaired renal excretion of sodium chloride. Am. J. M. Sc., 1942, **204**, 52.
2. Proger, S., Ginsberg, E., and Magendantz, H., The effects of the ingestion of excessive amounts of sodium chloride and water on patients with heart disease. Am. Heart J., 1942, **23**, 555.
3. Judson, W. E., Hatcher, J. D., Hollander, W., and Halperin, M. H., The effects of mitral valvuloplasty on cardiovascular and renal function at rest and during exercise. J. Clin. Invest., 1955, **34**, 1297.
4. Wilson, D. W., and Ball, E. G., A study of the estimation of chloride in blood and serum. J. Biol. Chem., 1928, **79**, 221.
5. Warren, J. V., and Stead, E. A., Jr., Fluid dynamics in chronic congestive heart failure; an interpretation of the mechanisms producing the edema, increased plasma volume and elevated venous pressure in certain patients with prolonged congestive failure. Arch. Int. Med., 1944, **73**, 138.
6. Fishman, A. P., Stamler, J., Katz, L. N., Miller, A. J., Silber, E. N., and Rubenstein, L., Mechanisms of edema formation in chronic experimental pericarditis with effusion. J. Clin. Invest., 1950, **29**, 521.
7. Davis, J. O., Hyatt, R. E., and Howell, D. S., Right-sided congestive heart failure in dogs produced by controlled progressive constriction of the pulmonary artery. Circ. Research, 1955, **3**, 252.
8. Relman, A. S., and Schwartz, W. B., The effect of DOCA on electrolyte balance in normal man and its relation to sodium chloride intake. Yale J. Biol. & Med., 1952, **24**, 540.
9. Deming, Q. B., and Luetscher, J. A., Jr., Bioassay of desoxycorticosterone-like material in urine. Proc. Soc. Exper. Biol. & Med., 1950, **73**, 171.
10. Parrish, A. E., The bioassay of adrenal corticoids in the urine of patients with congestive heart failure. J. Clin. Invest., 1949, **28**, 45.

A STUDY OF SERUM LIPOPROTEINS IN NORMAL AND ATHEROSCLEROTIC PATIENTS BY PAPER ELECTROPHORETIC TECHNIQUES

By WILLIAM P. JENCKS, MARION R. HYATT, MERA R. JETTON, THOMAS W. MATTINGLY, AND E. L. DURRUM [1]

(From the Department of Pharmacology, Walter Reed Army Institute of Research and the Department of Medicine, Walter Reed Army Hospital, Walter Reed Army Medical Center, Washington, D. C.)

(Submitted for publication December 5, 1955; accepted May 17, 1956)

The existence of a specific abnormality of lipid metabolism in patients with atherosclerosis has been suggested by recent studies which have shown that the concentration of certain serum lipoproteins in patients with coronary disease is statistically different from that found in "normal" individuals. Gofman and his co-workers (1) found in such patients an elevation of a beta lipoprotein sub-fraction defined by ultracentrifugal flotation rates between Sf 10 and 20. Russ, Eder, and Barr (2, 3) measured the cholesterol content of precipitated serum lipoprotein fractions and found that the total beta lipoprotein fraction was increased in serum from a group of atherosclerotic patients as compared to a control group made up largely of hospital personnel. Similar results have been obtained by Nikkilä (4), who measured the cholesterol content of electrophoretically separated protein fractions, and by Antonini *et al.* (5) and Kroetz and Fischer (6) who measured lipoprotein concentration in terms of the lipid dye binding of serum proteins separated by paper electrophoresis.

Studies on the validity and reproducibility of two relatively simple methods for the estimation of electrophoretically separated serum lipoproteins by measurement of their cholesterol content and dye binding capacity have recently been reported from this laboratory (7, 8). These methods are not subject to significant error due to loss of lipoprotein bound dye during alcohol rinsing, oxidation of cholesterol, or tailing of lipoprotein during electrophoresis similar to that found with albumin. The studies reported here were carried out in order to test the large scale application of these methods, to compare the results of the two methods with each other and with ultracentrifugal methods, and to confirm further the association of serum lipoprotein abnormalities with atherosclerosis, making use of both healthy and hospitalized control groups to lessen the possibility that any observed abnormalities might be the result of illness, medication, diet, or restricted activity rather than of atherosclerosis *per se*.

[1] Present address: Department of Pharmacology, Stanford University Medical School, Clay & Webster Sts., San Francisco 15, California.

MATERIALS AND METHODS

Separation and analysis of serum proteins and lipoproteins was carried out as previously described (7–10). Lipid material with zero mobility on paper, consisting of chylomicrons and adsorbed beta lipoprotein, was included in the beta lipoprotein fraction. A small amount of lipid staining material with a mobility slightly greater than beta lipoprotein was occasionally observed. Unpublished experiments from this laboratory have confirmed the finding of Ray, Davisson, and Crespi (11) that isolated beta lipoproteins are oxidized during dialysis and have also shown that this may occur during paper electrophoresis, resulting in an increase in the mobility of beta lipoprotein to that of an alpha$_2$ or even alpha$_1$ globulin. Although this may be prevented by ethylenediaminetetraacetic acid and is normally prevented in whole serum by the presence of albumin, the possibility that the observed lipid staining material may be a fraction of beta lipoprotein of slightly increased mobility as a result of oxidation (12) or binding of traces of fatty acid anions (13) has not been ruled out. This possibility is supported by the fact that this fraction usually occurs as a shoulder on the beta lipoprotein peak rather than as a separate fraction and by the wide range of mobility, ranging from beta to alpha$_2$ globulin, observed for the main lipoprotein band of many pathological sera. For these reasons, the distinguishing of a separate alpha$_2$ lipoprotein fraction did not appear to be warranted on the basis of evidence so far available and was not attempted in this study.

Oil Red O staining of separated lipoproteins was carried out with Lot No. 15448 (National Aniline Division, Allied Chemical and Dye Corp.). A rough, empirical estimate of the absolute concentrations of alpha and beta lipoproteins was obtained from this procedure by comparison of the dye uptake by lipoprotein to the dye uptake of a standard of 125 μg. of triolein applied to the paper strip in 0.025 cc. of chloroform and stained at the same

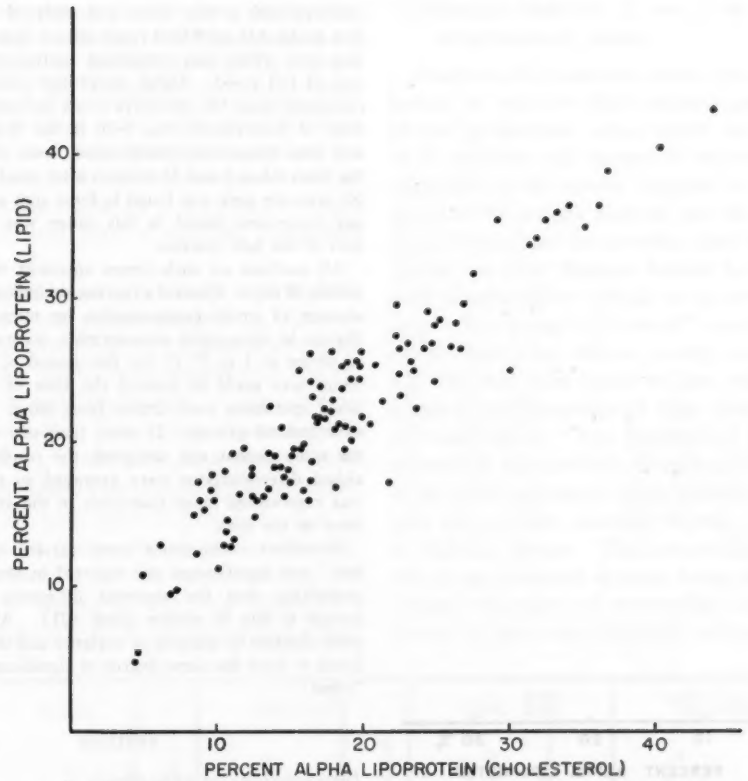


FIG. 1. THE RELATIONSHIP BETWEEN THE FRACTION OF THE TOTAL SERUM CHOLESTEROL FOUND IN THE ALPHA LIPOPROTEIN FRACTION AND THE FRACTION OF THE TOTAL LIPID DYE BINDING CAPACITY FOUND IN THE ALPHA LIPOPROTEIN FRACTION AS MEASURED BY DUPLICATE PAPER ELECTROPHORETIC DETERMINATIONS ON 126 NORMAL AND ABNORMAL SERA

time as the lipoprotein. Preliminary experiments indicated that the use of such a triolein standard resulted in as good or better agreement between replicate serum analyses as did the use of a standard serum (8).

Lipoprotein concentrations estimated by this method are reported arbitrarily in terms of "triolein units" which represent the amount of triolein in mg. per 100 cc. which would take up the same amount of dye as was taken up by the sample analyzed. It is not possible to express lipoprotein concentration as measured by this method in terms of absolute concentration because of the variation in the dye uptake of different lipids.

The sum of the alpha and beta lipoprotein cholesterol values after electrophoresis and elution was compared to the results of total cholesterol determinations by the Sperry and Webb modification of the Schoenheimer-Sperry procedure (14) on 126 normal and abnormal sera. The mean values for total cholesterol obtained by the two methods agreed within 2.2 per cent (standard deviation 8 per cent) indicating that no appreciable systematic error was influencing the lipoprotein cholesterol elutions and determinations.

Scanning of electrophoretically separated serum proteins stained with bromphenol blue was carried out with an automatically recording instrument (15) using tungsten light passed through a Bausch and Lomb 604 mμ second order interference filter, a Corning 3385 cut-off filter and a 2-mm. slit. The albumin values were corrected for the non-linear relationship between scanner response and high concentrations of dye on paper by reference to a standard curve obtained by elution of dye from the albumin and globulin fractions of normal and pathological sera. No correction was made for the 3 to 5 per cent loss of albumin due to adsorption on the paper (10) and no attempt was made to convert results to other units than dye binding; for this reason the values for protein distribution obtained by this method are not the same as those obtained by the moving boundary method when measured by increment in refractive index.

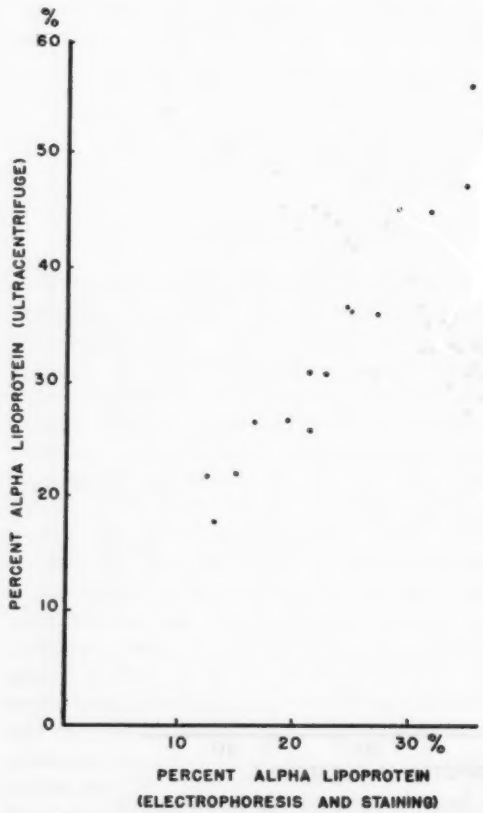


FIG. 2. A COMPARISON OF THE RATIO OF ALPHA TO TOTAL SERUM LIPOPROTEIN AS MEASURED BY PAPER ELECTROPHORESIS AND LIPID DYE BINDING TO THE RATIO OF ALPHA$_1$ ($Sf_{1.21}$ 0–20) TO TOTAL ($Sf_{1.21}$ 0–250) SERUM LIPOPROTEIN AS MEASURED BY ULTRACENTRIFUGATION AND REFRACTIVE INDEX INCREMENT

Total protein concentration was determined by the biuret method (16) using as a standard a solution of bovine albumin which was calibrated by micro Kjeldahl determination of nitrogen using the factor 6.25 to convert nitrogen to protein concentration.

Ultracentrifugal analysis of lipoproteins by flotation at a density of 1.063 was carried out as described (17). Ultracentrifugation at a density of 1.21 was carried out by a modification of the methods of Lewis and Page (18), de Lalla and Gofman (19) and Boyle, Bragdon, and Brown (20).[2] Preparative ultracentrifugation was carried out in a KBr—NaCl medium of density 1.21 at 16° C. for 18 hours at 52,640 rpm in the J rotor of the Spinco Model E ultracentrifuge. The top fraction was removed with a tube slicer and analyzed at 56,100 rpm in a model AD analytical rotor using a 6-minute acceleration time which was considered equivalent to two minutes at full speed. Alpha$_1$ lipoprotein concentration was calculated from the refractive index increment of lipoproteins of uncorrected $Sf_{1.21}$ 0–20 in the 40-minute picture and beta lipoprotein concentration from $Sf_{1.21}$ 20–250 in the films taken 2 and 12 minutes after reaching full speed. No separate peak was found in these sera at Sf 20–25 and any lipoprotein found in this range was included as a part of the beta fraction.

All analyses on each serum specimen were completed within 30 days. Control experiments indicated that in the absence of gross contamination by microorganisms no change in lipoprotein concentration occurred on storage of serum at 1 to 3° C. for this period of time. No attempt was made to control the time of day at which blood specimens were drawn from either the control or experimental groups. If more than one specimen from the same patient was analyzed, the results of the individual determinations were averaged so that no patient was represented more than once in the statistical evaluation of the data.

Statistical comparisons were carried out by the "t test," and significances are reported in terms of "p," the probability that the observed difference between two groups is due to chance alone (21). All comparisons were checked by analysis of variance and the "F test" and found to have the same degree of significance as with the "t test."

RESULTS

Interrelationship of methods

In order to compare the results of the lipid staining with the cholesterol elution procedure for measuring the concentration of separated lipoproteins, analyses were carried out in duplicate by both of these methods on 126 normal and abnormal sera. The results are shown in Figure 1, expressed as the per cent of the total stainable lipid or cholesterol found in the alpha lipoprotein fraction of each serum. The two methods gave comparable results, with some deviation from linearity in their relationship because of the nonlinear dye uptake of high concentrations of beta lipoprotein (8). The fact that higher values are obtained for the per cent alpha lipoprotein in terms of stainable lipid than in terms of cholesterol content reflects the different composition of these lipoproteins; however, the fact that the results of the empirical and chemically non-specific staining procedure (which does not stain pure cholesterol) are at all comparable to those obtained by cholesterol analysis indicates a considerable degree of

[2] We are grateful to Dr. Edwin Boyle for advice on the analysis of lipoproteins at a density of 1.21 and for unpublished details for the construction of a tube slicer.

constancy in the composition of these lipoproteins from one serum to another. Comparisons of the absolute amounts of dye uptake with the cholesterol content of the individual lipoprotein fractions confirmed these conclusions, although these curves showed a slightly greater scatter than the curve of lipoprotein distribution because the staining method is more accurate as a measure of lipoprotein distribution than of absolute lipoprotein concentration (8).

A similar comparison between the results of the staining method and results obtained by ultracentrifugation at a density of 1.21 is shown in Figure 2. Comparable results were obtained for the ratio of alpha to total lipoprotein as measured by these two methods. The higher values for this ratio obtained by refractive index measurement in the ultracentrifugal procedure may be attributed to the relatively high ratio of protein to lipid in alpha lipoprotein (12). Again, the results suggest that, at least in the 16 sera examined, the composition of the alpha and beta lipoproteins does not vary greatly from one serum to another.

Lipoprotein analyses of sera from control and atherosclerotic groups

Analyses of lipoprotein concentration and distribution by both the lipid staining and cholesterol elution procedures were carried out on a group of 77 patients with presumed atherosclerosis and compared to the results obtained on sera from a group of 68 healthy controls and 65 patients who were hospitalized for reasons other than vascular disease or other diseases known to be associated with abnormalities in lipid or lipoprotein metabolism. The group of "normal" controls consisted of 68 male Army officers, mostly over the age of 45, who had been found to have no clinical evidence of cardiovascular or other disease on physical examination. The hospitalized patients were selected at random from those hospital admissions in the same age range as the atherosclerotic group who did not have vascular, hepatic, renal, thyroid or diabetic disease. The presumably atherosclerotic group consisted of male Army personnel with clinical diagnoses of myocardial infarction supported by electrocardiographic evidence.

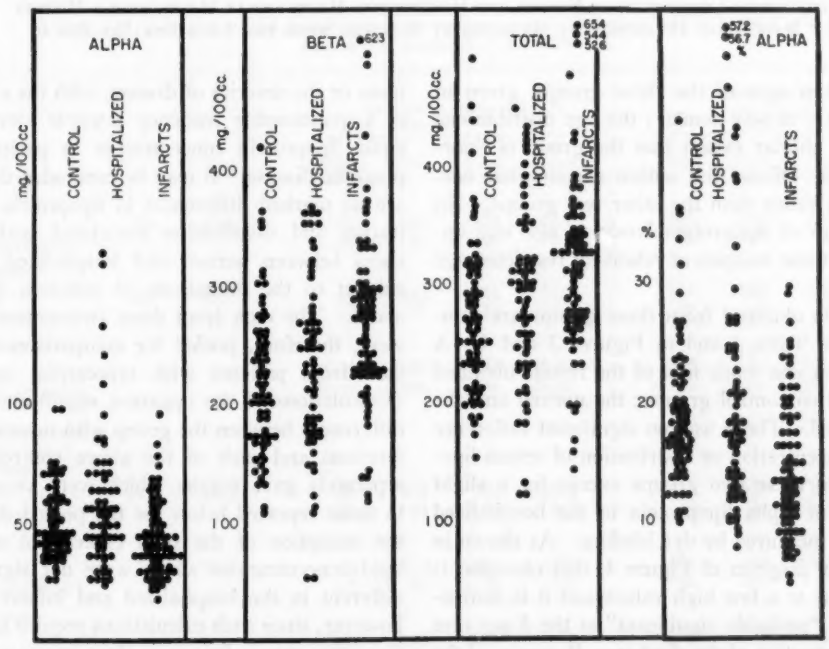


FIG. 3. SCATTER DIAGRAMS SHOWING THE CONCENTRATION AND DISTRIBUTION OF SERUM LIPOPROTEIN CHOLESTEROL IN NORMAL AND HOSPITALIZED MALES AND IN MALES WITH A HISTORY OF MYOCARDIAL INFARCTION

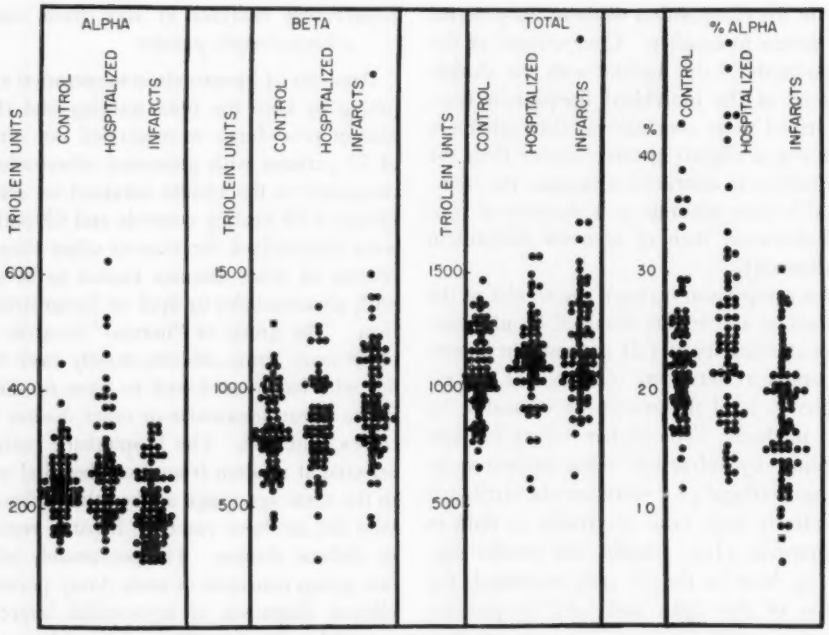


FIG. 4. SCATTER DIAGRAMS SHOWING THE CONCENTRATION AND DISTRIBUTION OF LIPIDS IN THE SERUM LIPOPROTEINS OF NORMAL AND HOSPITALIZED MALES AND IN MALES WITH A HISTORY OF MYOCARDIAL INFARCTION AS MEASURED BY STAINING WITH THE LIPID DYE OIL RED O

The mean ages of the three groups, given in Table I, are closely similar; the age distributions were also similar except that the group of "normal" Army officers fell within a somewhat narrower age range than the other two groups. No relationship of lipoprotein levels to age was apparent in these samples of relatively restricted age range.

The data obtained from these groups are summarized in Table I and in Figures 3 and 4. A comparison was made first of the results obtained with the two control groups: the normal and the hospitalized. There was no significant difference in the concentration or distribution of serum lipoproteins in these two groups except for a slight elevation of alpha lipoprotein in the hospitalized group, as measured by dye binding. As shown in the scatter diagram of Figure 4, this elevation is largely due to a few high values and it is statistically only "probably significant" at the 5 per cent level. Inspection of the discharge diagnoses of the patients in the hospitalized group revealed no correlation of lipoprotein abnormalities with the diagnosis or the severity of disease, with the exception of a questionable tendency towards elevation of alpha lipoprotein concentration in patients with prostatic disease. It may be concluded that there are no marked differences in lipoprotein concentration and distribution associated with differences between normal and hospitalized patients subject to the limitations of selection described above. The data from these two control groups were, therefore, pooled for comparison with the data from patients with myocardial infarction. (Calculations of the apparent significance of the differences between the group with myocardial infarctions and each of the above control groups separately gave results which were very similar to those reported below for the pooled data, with the exception of the total cholesterol and total lipid concentrations which were not significantly different in the hospitalized and infarct groups; however, since such calculations require the use of the same group for more than one comparison, they are not independent comparisons and are, therefore, not reported in detail.)

The data obtained on patients with myocardial infarctions (Table I) indicate that this group differs from the pooled control group in all measures of lipid and lipoprotein concentration and distribution that were studied, regardless of the method used for analysis. The differences are all statistically significant at the 0.1 per cent level except for the increase in total cholesterol which is significant only at the 1.0 per cent level and the increase in total lipid which is probably significant at the 5 per cent level. It is evident from the results of both the cholesterol and the staining methods that the concentration of alpha lipoprotein is decreased and the concentration of beta lipoprotein is increased in the coronary group. The ratio of alpha to total lipoprotein, or "per cent alpha," which is a function of both of these changes, therefore shows an even greater difference between the groups than either the alpha or the beta fraction alone. The distribution and overlap of the individual values obtained in each group are shown in Figures 3 and 4.

A quantitative measure of the relative effectiveness of the different measurements made on sera from control and atherosclerotic groups in differentiating these groups from each other was obtained by calculation of the value r^2 from the formula $r^2 = \frac{t^2}{n - 2 + t^2}$ (21, p. 193).[3] The value so obtained represents the fraction of the total variation in a set of measurements which is due to differences between the groups rather than to random variation within each group; thus, a large value for r^2 indicates that a particular measurement differentiates two groups effectively, while a small value indicates that random variation of that measurement is large compared to the difference between the two groups. The relatively low values for r^2 shown in Table I reflect the overlap of measurements in the control and atherosclerotic groups. However, it is evident that measurement of either alpha or beta lipoprotein concentration is a considerably better means for differentiating these

[3] We are greatly indebted to Dr. Ardie Lubin of the Department of Psychology, Walter Reed Army Institute of Research, for suggesting this method of analysis.

TABLE I

Lipoprotein concentration and distribution in normal and hospitalized males and in patients with a diagnosis of myocardial infarction

			Lipoprotein cholesterol (*mg./100 cc.*)				Lipoprotein dye-binding triolein units (*mg./100 cc.*)			
	n	Age	Alpha	Beta	Total	% Alpha	Alpha	Beta	Total	% Alpha
Normal:										
Mean	68	47.8	51	223	274	19.4	246	840	1,086	22.7
Std. deviation			16	63	68	6.6	58	179	183	6.0
Hospitalized:										
Mean	65	50.9	58	233	291	21.0	273	866	1,140	24.4
Std. deviation			29	75	76	11.4	97	315	296	8.5
Comparison										
"t"			1.64	0.80	1.35	0.97	2.02	0.62	1.27	1.18
Significance (p)			None	None	None	None	<5%	None	None	None
Pooled normal and hospitalized controls:										
Mean	133	49.3	55	228	282	20.2	259	853	1,112	23.6
Std. deviation			24	69	72	9.3	80	254	245	7.4
Infarcts:										
Mean	77	50.4	43	273	316	14.3	208	986	1,195	17.9
Std. deviation			16	81	81	5.8	64	265	260	5.5
Comparison										
"t"			3.85	4.27	3.10	5.00	4.75	3.63	2.32	6.02
Significance (p)			<0.1%	<0.1%	<1.0%	<0.1%	<0.1%	<0.1%	<5%	<0.1%
r^2			0.067	0.081	0.044	0.107	0.098	0.060	0.025	0.148

TABLE II

Average distance from the point of application to the center of the peaks of electrophoretically separated alpha and beta lipoproteins

	Distance of migration (cm.)	
	Alpha	Beta
Normal controls	3.34	0.86
Hospitalized	3.15	0.86
Infarcts	3.24	0.85

groups, regardless of whether the measurement is carried out by staining or cholesterol elution, than is measurement of either total cholesterol or total lipid. The ratio of alpha to total lipoprotein is, as might be expected, the best index of difference between the groups, since it reflects the differences in both alpha and beta lipoproteins.

A comparison of serum lipoprotein distributions at varying periods of time after myocardial infarction revealed some tendency towards an increase in the per cent alpha lipoprotein six months or more after an acute episode. The average value for the per cent of the total cholesterol in the alpha lipoprotein fraction of sera drawn within one month after an acute attack was 14.7 per cent, which was not significantly different from the value of 12.4 per cent obtained on sera drawn between one and six months after an attack. However, sera drawn over six months after an attack had an alpha lipoprotein fraction of 16.1 per cent which is probably significantly greater than the combined values for 0 to 6 months ($p = < 5$ per cent). Whether this increase indicates that the abnormality in lipoprotein distribution found in patients with myocardial infarction is a transient metabolic alteration which returns to normal with time or whether it is a result of selection due to death of those individuals with low values within a six-month period after their first infarction cannot be decided from these data. However, the second alternative appears somewhat more likely since after the first month, during which patients might be expected largely to recover from the acute effects of their illness, there is no increase, but actually a small decrease in the per cent alpha lipoprotein.

If the lipoproteins in atherosclerotics are qualitatively as well as quantitatively different from normal, one manifestation of this abnormality could be a shift in electrophoretic mobility. The mobility of the lipoproteins of the control and atherosclerotic groups was estimated by measuring the distance between the point of serum application and the peak of each stained lipoprotein fraction before elution. Such a procedure, while not very accurate in any single determination, should show any tendency towards abnormal mobility in a large group of samples. The results shown in Table II indicate that no such abnormality of lipoprotein mobility occurred in this group of patients.

The electrophoretic distribution of serum proteins expressed in terms of bromphenol blue dye binding capacity in normal, hospitalized and infarction groups is shown in Table III. The average concentration of albumin is slightly lower, and all of the globulin fractions are slightly higher in the hospitalized compared with the normal group. This relatively non-specific alteration in serum protein distribution is to be expected as a result of illness in this group of patients (22). The group of patients with myocardial infarction, on the other hand, shows only a very small decrease in albumin accompanied by a small generalized rise in the globulin fractions. It appears likely that this small change, if significant at all, is a non-specific response to illness and is in contrast to the abnormalities of lipoprotein distribution found in this group of patients, which were not found in the hospitalized group. These results may be compared to the results of other studies from this and other laboratories (22, 23) in which considerably more marked decreases in albumin concentration were observed in atherosclerotic patients. It seems likely that this difference may also be accounted for by differences in the degree of illness in the various groups: the group of atherosclerotic patients to be reported elsewhere were

TABLE III

Serum protein distribution in normal and hospitalized males and in patients with myocardial infarction as measured by paper electrophoresis

	Albumin (%)	Alpha$_1$ globulin (%)	Alpha$_2$ globulin (%)	Beta globulin (%)	Gamma globulin (%)	Total protein (gm./100 cc.)
Controls	66.8	3.3	7.5	10.2	12.2	6.8
Hospitalized	62.3	3.9	9.1	11.2	13.5	7.1
Infarcts	65.5	3.4	8.1	10.6	12.5	6.9

all hospitalized and many of them were critically ill.

In spite of the increased concentration of beta lipoprotein in the patients with coronary atherosclerosis, the relative concentration of beta globulin is not appreciably increased; the very small increase observed is accompanied by similar increases in the other globulins and may simply reflect the small fall in albumin concentration. Plots of alpha and beta lipoprotein against $alpha_1$ and beta globulin concentrations respectively in each serum failed to reveal any relationship between lipoprotein and globulin concentration in either normal or abnormal sera. It was concluded that it was not possible to infer the presence of abnormal lipoprotein levels from the results of paper electrophoresis of serum without specifically measuring the concentrations of the lipoproteins (this may not necessarily be the case in moving boundary electrophoresis in which lipid makes a considerable contribution to the measured concentration of beta globulin, so that lipoprotein accounts for 15 to 30 per cent of the beta globulin peak in normal sera and up to 60 per cent in abnormal sera when measured by refractive index increment [18]). Since the lipoproteins represent only a small fraction of the total alpha and beta globulins of serum, it is also not possible to draw any conclusions about the ratio of lipid to protein in the lipoprotein molecules of atherosclerotic individuals from the type of data reported here.

Comparison of ultracentrifugal with electrophoretic procedures in normal and atherosclerotic groups

A comparison was made of the results obtained by analyzing 97 sera by ultracentrifugation at a density of 1.063 according to the technique developed by Gofman and his associates (1) with the results of analyses on the same sera by paper electrophoresis. In Figure 5 the concentration of the beta lipoprotein subfraction with uncorrected flotation rates of Sf 12–20 is plotted against the per cent of the total cholesterol in the alpha lipoprotein fraction, for each of the 68 normal and the 29 atherosclerotic (as judged by either myocardial infarction or angina pectoris) individuals in this group. No quantitative comparison of the relative

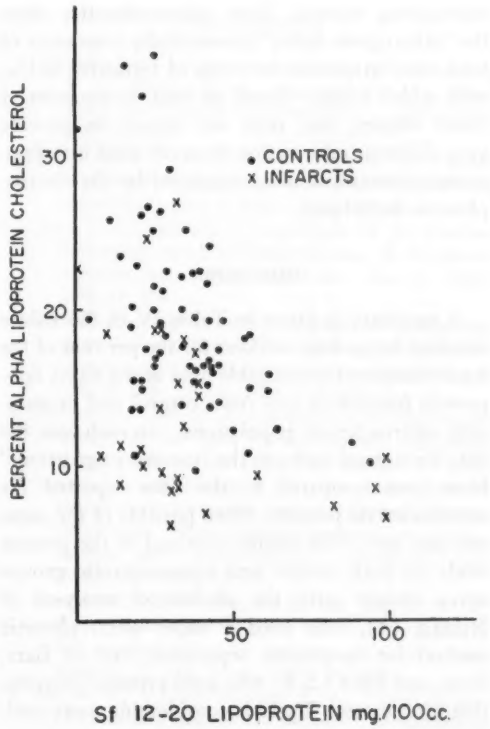


FIG. 5. THE CONCENTRATION OF SERUM LIPOPROTEIN WITH FLOTATION RATES OF SF 12–20 AT A DENSITY OF 1.063 AND THE PER CENT OF THE TOTAL SERUM CHOLESTEROL FOUND IN THE ALPHA LIPOPROTEIN FRACTION IN A GROUP OF NORMAL MALES AND A GROUP OF MALES WITH A HISTORY OF MYOCARDIAL INFARCTION OR ANGINA PECTORIS

effectiveness of the two procedures in segregating normal from atherosclerotic populations was attempted with this number of analyses, but it is quite apparent from inspection of the data that the electrophoretic procedure is considerably more effective than this particular ultracentrifugal measurement in this respect; *i.e.*, although with both procedures there is a considerable overlap of the values for the normal and control groups, the per cent alpha lipoprotein cholesterol is much more often outside the normal range in atherosclerotic patients than is Sf 12–20 lipoprotein.

No attempt was made to compare the relative effectiveness of the "atherogenic index" (24) with the results of the electrophoretic procedures in

segregating normals from atherosclerotics since the "atherogenic index" is essentially a measure of total beta lipoprotein in terms of refractive index, with added weight placed on beta lipoproteins of lower density, and does not appear to provide very different information from the total beta lipoprotein concentration as measured by the electrophoretic techniques.

DISCUSSION

A summary is given in Table IV of the values obtained by various workers for the per cent of the total cholesterol or stainable lipid in the alpha lipoprotein fraction of sera from control and presumably atherosclerotic populations. In each case the data for normal males in the "coronary age group" have been compared to the data reported for atherosclerotic patients, when possible of the same sex and age. The results obtained in the present study on both control and atherosclerotic groups agree closely with the cholesterol analyses of Nikkilä (4), who used a paper electrophoretic method for lipoprotein separation, and of Barr, Russ, and Eder (2, 3) who used protein precipitation procedures. There is considerably more variation in the results of different laboratories when lipoprotein concentration is measured by lipid dye binding, although the results of published studies agree in the finding that the fraction of alpha lipoprotein is lowered in atherosclerotic populations. This variation is not unexpected in view of the different staining techniques used in different laboratories, and serves to emphasize the necessity for careful evaluation of the validity and reproducibility of the procedure itself, and the analysis of control sera at the same time and under the same conditions as experimental sera if meaningful results are to be obtained with such techniques.

TABLE IV

A summary of data from various sources on the distribution of serum lipoproteins from "normal" and atherosclerotic individuals

	Per cent alpha lipoprotein	
	Normal	Atherosclerosis
	%	%
Cholesterol		
Barr (27) (% cholesterol in fraction A)	22.9	14.1
Nikkilä (4) (% cholesterol in albumin and alpha$_1$ globulin)	20.8	14.6
Present Study		
Normals	19.4	14.3
Hospitalized	21.0	
Stain		
Kroetz and Fischer (6) (% stain in albumin and alpha$_1$ globulin)	34.0	22.5
Antonini *et al.* (5)	25.0	18.5
Present Study		
Normals	22.7	17.9
Hospitalized	24.4	

The most serious difficulties encountered in studies of the relationship of lipoprotein levels to atherosclerosis are the absence of a method to determine the presence or absence of atherosclerosis in the "normal" subject during life and the difficulty of selecting control and experimental groups which differ *only* in their incidence of atherosclerosis. It is probably reasonable to assume that a group of patients with proved myocardial infarction will have definite atherosclerosis (25), but the incidence of atherosclerosis in clinically normal males of comparable age, which is probably well over 50 per cent (26), means that any measurement which is in fact associated with atherosclerosis will show a considerable overlap between control and atherosclerotic populations. The data obtained in the present study are shown in the form of scatter diagrams in Figures 3 and 4 to emphasize this overlap, which is not immediately apparent from inspection of the mean values and the statistical significance of their differences (Table I). That the observed differences between control and atherosclerotic groups are in fact associated with atherosclerosis and not some other difference between the groups is strongly suggested, although still not conclusively proven, by the finding that significant alterations of lipoprotein levels did not occur in a control group of patients hospitalized for illnesses not associated with atherosclerosis. Finally, it should be kept in mind that a correlation between any two variables, such as atherosclerosis and the level or distribution of serum lipoproteins, provides no proof of any casual relationship between the variables, but can only serve as an indication of an association between them and suggest paths for further investi-

gation of abnormal metabolic pathways to establish any causal relationship.

SUMMARY

1. A standardized staining technique for the estimation of serum lipoproteins separated by paper electrophoresis gave results which were comparable to the results of cholesterol determinations on these lipoproteins and to the results of ultracentrifugal analysis of lipoproteins at a density of 1.21.

2. No appreciable difference in lipoprotein concentration or distribution, measured by paper electrophoretic techniques, was found between the sera of 68 healthy male Army officers and the sera of 65 patients hospitalized with diseases not known to be associated with atherosclerosis or abnormalities in lipid metabolism.

3. Analyses of sera from 77 males with atherosclerosis, as judged by a history of myocardial infarction, showed a significant drop in alpha lipoprotein and increase in beta lipoprotein, resulting in a decreased ratio of alpha to total lipoprotein in these sera as compared to the control groups. Elevations in total cholesterol and total lipid dye binding capacity were less significant.

4. The percentage of the total serum cholesterol in the alpha lipoprotein fraction was lower in sera from patients studied from 0 to 6 months than in those studied over 6 months after an acute episode of myocardial infarction.

5. The electrophoretic distribution of serum proteins showed greater deviations from normal in the group of hospitalized patients than in the atherosclerotic group.

6. Ultracentrifugal analyses of lipoproteins of Sf 12–20 were less often abnormal than were electrophoretic analyses of the ratio of alpha to total lipoprotein in sera from atherosclerotic individuals.

ACKNOWLEDGMENTS

The authors are greatly indebted to the Medical Service of Walter Reed Army Hospital, the Base Hospital of the Bolling Air Force Base, the U. S. Army Hospital at Fort Belvoir and the Dispensary at the Pentagon for their cooperation in providing serum specimens, and to Mr. Fredoon Nury, Mr. Clarence Lockett, and Mr. Albert Lafayette for technical assistance in various phases of this investigation.

REFERENCES

1. Gofman, J. W., Jones, H. B., Lindgren, F. T., Lyon, T. P., Elliott, H. A., and Strisower, B., Blood lipids and human atherosclerosis. Circulation, 1950, **2**, 161.
2. Russ, E. M., Eder, H. A., and Barr, D. P., Protein-lipid relationships in human plasma. I. In normal individuals. Am. J. Med., 1951, **11**, 468.
3. Barr, D. P., Russ, E. M., and Eder, H. A., Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. Am. J. Med., 1951, **11**, 480.
4. Nikkilä, E., Studies on the lipid-protein relationships in normal and pathological sera and the effect of heparin on serum lipoproteins. Scandinav. J. Clin. & Lab. Invest., 1953, **5**, Suppl. 8.
5. Antonini, F. M., *et al.* Lipoproteine ed eparina nel quadro umorale della chemiopatogenesi dell' aterosclerosi. Giorn. Gerontol., 1953, Suppl. 1.
6. Kroetz, C., and Fischer, F. W., Zur Blutchemie der akuten fortschreitenden Arteriosklerose. Elektrophoretische Lipoproteinbestimmungen bei Atheromatose und Atherosklerose. Deutsche med. Wchnschr., 1954, **79**, 653.
7. Langan, T. A., Durrum, E. L., and Jencks, W. P., Paper electrophoresis as a quantitative method: Measurement of alpha and beta lipoprotein cholesterol. J. Clin. Invest., 1955, **34**, 1427.
8. Jencks, W. P., and Durrum, E. L., Paper electrophoresis as a quantitative method: The staining of serum lipoproteins. J. Clin. Invest., 1955, **34**, 1437.
9. Williams, F. G., Jr., Pickels, E. G., and Durrum, E. L., Improved hanging-strip paper-electrophoresis technique. Science, 1955, **121**, 829.
10. Jencks, W. P., Jetton, M. R., and Durrum, E. L., Paper electrophoresis as a quantitative method. Serum proteins. Biochem. J., 1955, **60**, 205.
11. Ray, B. R., Davisson, E. O., and Crespi, H. L., Experiments on the degradation of lipoproteins from serum. J. Phys. Chem., 1954, **58**, 841.
12. Oncley, J. L., and Gurd, F. R. N., The lipoproteins of human plasma *in* Tullis, J. L., Blood Cells and Plasma Proteins. Their State in Nature. New York, Academic Press, 1953, p. 337.
13. Gordon, R. S., Jr., Interaction between oleate and the lipoproteins of human serum. J. Clin. Invest., 1955, **34**, 477.
14. Sperry, W. M., and Webb, M., A revision of the Schoenheimer-Sperry method for cholesterol determination. J. Biol. Chem., 1950, **187**, 97.
15. Durrum, E. L., and Gilford, S. R., Recording integrating photoelectric and radioactive scanner for paper electrophoresis and chromatography. Rev. Scient. Instruments, 1955, **26**, 51.
16. Gornall, A. G., Bardawill, C. J., and David, M. M., Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 1949, **177**, 751.
17. U. S. National Advisory Heart Council. Technical Group of the Committee on Lipoproteins and

Atherosclerosis, Lipoproteins and ultracentrifugal technique, a Symposium, 1952.

18. Lewis, L. A., and Page, I. H., Electrophoretic and ultracentrifugal analysis of serum lipoproteins of normal, nephrotic, and hypertensive persons. Circulation, 1953, **7**, 707.
19. de Lalla, O. F., and Gofman, J. W., Ultracentrifugal analysis of serum lipoproteins. Methods of Biochemical Analysis, 1954, **1**, 459.
20. Boyle, E., Bragdon, J. H., and Brown, R. K., Role of heparin in *in vitro* production of alpha$_1$ lipoproteins in human plasma. Proc. Soc. Exper. Biol. & Med., 1952, **81**, 475.
21. Fisher, R. A., Statistical Methods for Research Workers. 11th ed., Edinburgh, Oliver and Boyd, 1950.
22. Jencks, W. P., Smith, E. R. B., and Durrum, E. L., The clinical significance of the analysis of serum protein distribution by filter paper electrophoresis. Am. J. Med., In press.
23. Leinwand, I., and Moore, D. H., Serum lipid and protein fractions. IX. Comparisons of ninety-six patients with vascular disease and sixty normal controls (with additional notes on blood donors). Circulation, 1954, **10**, 94.
24. Gofman, J. W., Glazier, F., Tamplin, A., Strisower, B., and de Lalla, O., Lipoproteins, coronary heart disease, and atherosclerosis. Physiol. Rev., 1954, **34**, 589.
25. Yater, W. M., Traum, A. H., Brown, W. G., Fitzgerald, R. P., Geisler, M. A., and Wilcox, B. B., Coronary artery disease in men eighteen to thirty-nine years of age. Report of eight hundred sixty-six cases, four hundred fifty with necropsy examinations. Am. Heart J., 1948, **36**, 334, 481, and 683.
26. Enos, W. F., Holmes, R. H., and Beyer, J., Coronary disease among United States soldiers killed in action in Korea. Preliminary report. J. A. M. A., 1953, **152**, 1090.
27. Barr, D. P., The George E. Brown Memorial Lecture. Some chemical factors in the pathogenesis of atherosclerosis. Circulation, 1953, **8**, 641.

CARBOHYDRATE METABOLISM IN INTACT SKELETAL MUSCLE IN MAN DURING THE NIGHT [1]

By KENNETH L. ZIERLER AND REUBIN ANDRES

(From the Departments of Environmental Medicine and Medicine, The Johns Hopkins University and Hospital, Baltimore, Md.)

(Submitted for publication March 20, 1956; accepted May 17, 1956)

It is customary in many metabolic studies to reduce variables by limiting observations to the so-called basal state in which the subject is not only at rest but also has been fasted for 14 hours or more. Despite the usefulness of this standardization it must be remembered that the basal state is unique; for example, during man's waking hours no more than four to six hours elapse between meals.

In a previous study (1) it was shown that in normal man in the basal state resting skeletal muscle (exemplified by forearm tissues) oxidizes noncarbohydrate substrates, in the main, and despite vigorous oxygen consumption, produces lactate. Since these results ran counter to concepts held generally it was conceivable that they were characteristic only of early starvation since they were obtained between the hours of 10 A.M. and noon, that is, 16 to 18 hours after the last meal. To examine this possibility observations of forearm metabolism in three normal men have been made throughout the night, beginning four to six hours after the evening meal and continuing through the period defined previously as the basal state. Measurements were made of O_2 and glucose uptake and CO_2 and lactate production by application of the Fick principle, $\dot{Q} = F\ (A\text{-}V)$, where $\dot{Q}$ is rate of uptake or production, F is blood flow, and A and V are concentrations in arterial and in venous blood, respectively.

METHODS

Normal subjects were three fourth-year medical students in good health. They ate their usual evening meal at about 6 P.M. and reported to the laboratory between three and four hours later. They were immediately put to bed and made as comfortable as possible. An 18-gauge Riley needle was inserted in a brachial artery and a deep and a superficial antecubital vein were catheterized as described previously (2). Intravenous catheters were kept patent between sampling periods by slow infusion of 155 mM NaCl solution at a rate of approximately 20 ml. per hr. Eight or nine pairs of arterial and venous samples were collected through the night and during the following morning. During this time the subjects had neither food nor drink. Two of them fell asleep shortly after the procedure started and had to be awakened in the morning. The third, subject N, slept only fitfully and complained so bitterly of pain in his arm that he had to be given codeine, 32 mg., at 3 A.M. and again at 3:50 A.M. At least five minutes before each collection period a sphygmomanometer cuff about the wrist was pumped to at least 200 mm. Hg pressure to exclude the hand from the forearm circulation and intra-arterial injection of T-1824 at constant rate was instituted to give a measure of blood flow as described previously (2). The cuff was released and the injection discontinued immediately after collection of each pair of blood samples. Each blood sample was 18 ml. in volume, collected in 20-ml. syringes, the dead space of which was filled with heparin. Handling of blood and analytical methods were as previously described (1, 2). Venous concentration of metabolites was determined only in blood from the deep vein draining muscle. To discover whether or not venous blood was diluted by backflow of the infused NaCl solution hematocrits were determined on each sample. No such contamination was detected.

RESULTS

Results appear in Table I A, B and C and in Table II.

Blood flow

There was no evidence of diurnal variation in blood flow. Although there is no way of knowing whether or not there were rapid changes of great magnitude in flow at times not sampled, if these were absent, the changes in flow as measured occurred over an hour or two and the change in flow per minute was probably too small to jeopardize the validity of application of the Fick principle which, of course, depends upon the existence

[1] This work was performed under a contract between the Office of Naval Research, Department of the Navy, and The Johns Hopkins University (NR 113-241), and was further supported by grants-in-aid from the National Institutes of Health, Department of Health, Education and Welfare (H-1327, A-750) and the Muscular Dystrophy Associations of America, Inc.

TABLE I

Blood flow and metabolism of the forearm during the night *

Time	Blood flow	Oxygen A	Oxygen (A-V)	Oxygen Q̇	CO_2 A	CO_2 (A-V)	CO_2 Q̇	Glucose A	Glucose (A-V)	Glucose Q̇	Lactate A	Lactate (A-V)	Lactate Q̇
A. Subject C													
11:47 P.M.	4.35	8.77	5.60	24.4	21.68	−3.91	17.0	5.00	0.30	1.30	0.452	−0.072	−0.313
1:03 A.M.	4.13	8.45	4.47	18.5	21.80	−3.38	14.0	5.20	0.25	1.03	0.442	−0.109	−0.450
2:03	5.15							5.11	0.16	0.82	0.405	−0.114	−0.587
3:03	4.41	8.27	3.48	15.4	21.64	−2.92	12.9	5.01	0.01	0.04	0.463	−0.068	−0.300
5:03	6.92	8.31	4.20	29.1	21.07	−3.51	24.3	4.82	0.13	0.90	0.464	−0.075	−0.519
7:03	3.23	8.21	3.70	11.9	21.27	−3.11	10.0	4.74	0.01	0.03	0.447	−0.101	−0.326
9:03	3.94	7.95	4.15	16.3	21.10	−3.56	14.0	4.74	0.07	0.28	0.493	−0.104	−0.410
10:03	6.30							4.73	0.01	0.06	0.479	−0.154	−0.970
11:03	3.23	8.54	3.69	11.9	20.31	−3.20	10.3	4.67	−0.09	−0.29	0.509	−0.119	−0.384
B. Subject K													
11:56 P.M.	3.34	9.79	3.07	10.25	21.58	−2.16	7.21	4.99	0.05	0.17	0.610	−0.128	−0.428
1:05 A.M.	3.53	9.58			21.62			4.87			0.486		
2:00	3.00	9.60	2.53	7.59	21.82	−2.04	6.12	4.79	0.17	0.51	0.533	−0.176	−0.528
3:33	3.39	9.44	2.50	8.48	21.23	−2.55	8.64	5.02	0.52	1.76	0.583	−0.169	−0.573
5:03	2.84	9.51	2.37	6.73	21.72	−1.83	5.20	4.67	0.14	0.40	0.562	−0.256	−0.727
6:34	2.36	9.49	3.06	7.22	22.18	−2.53	5.97	4.91	0.48	1.13	0.598	−0.328	−0.774
8:04	3.09	9.45	2.66	8.22	22.23	−2.86	8.84	4.79	0.20	0.62	0.597	−0.318	−0.983
10:16	3.62	9.71	2.36	8.54	22.20	−2.19	7.93	4.70	−0.19	−0.69	0.563	−0.281	−1.017
10:37	3.20	9.84	2.84	9.09	22.34	−2.14	6.85	4.80	−0.02	−0.06	0.565	−0.259	−0.829
11:04	3.37	9.69	2.04	6.87	21.90	−2.31	7.78	4.95	0.09	0.30	0.543	−0.272	−0.917

Time	Oxygen A	Oxygen (A-V)	CO_2 A	CO_2 (A-V)	Glucose A	Glucose (A-V)	Lactate A	Lactate (A-V)
C. Subject N								
10:08 P.M.	9.16	2.03	21.70	−1.98	5.32	0.56	0.583	−0.295
11:35	9.04	2.06	20.93	−1.91	5.53	0.30	0.663	−0.113
1:04 A.M.	8.82	2.55	21.13	−2.01	5.28	0.50	0.601	−0.084
3:04	9.03	2.24	20.86	−1.53	4.89	−0.10	0.672	+0.098
5:08	8.86	2.21	21.44	−1.09	4.52	−0.19	0.604	−0.028
7:07	8.87	2.02	20.86	−1.67	4.67	0.19	0.651	−0.125
8:04	8.91	2.21	20.85	−1.65	4.86	0.30	0.595	−0.246
9:09	9.09	2.62	21.00	−1.56	4.80	0.25	0.611	−0.090

* Time refers to time at which arterial blood was sampled.

Units: Blood flow, ml./min./100 ml. forearm; A = arterial concentration, mM/liter whole blood; (A-V) = arterial-venous concentration difference, mM/liter; Q = uptake or outflow, μM/min./100 ml. forearm. All values for Q_{CO_2} are negative.

of a steady state. In subject N it was possible to sample only from the deep vein. Since the method for measuring blood flow cannot be validated unless mixing of T-1824 and blood is demonstrated by analysis of blood from two veins, no data for blood flow are reported for this subject.

O_2 uptake

Oxygen uptake, although quite variable, did not change systematically with time. In general, A-V differences in O_2 content were more constant than O_2 uptake. In one subject O_2 uptake varied linearly with blood flow; in the other, neither flow nor O_2 uptake varied sufficiently to make it appropriate to test for correlation.

CO_2 production

There was no temporal trend in CO_2 production. Arterial CO_2 concentrations were quite constant.

Glucose uptake

Arterial glucose concentration decreased with time (Figure 1). Although in one subject, K, no clear-cut trend is evident, the association of decreasing arterial concentration with increasing time is excellent when the whole body of data is tested ($P < 0.01$). There was a similar trend toward decreasing A-V differences in glucose concentration and toward decreasing glucose uptake. Indeed a high correlation ($p < 0.01$) exists

TABLE II

Per cent of glucose uptake accounted for by lactate production and per cent of O_2 uptake accounted for by glucose oxidation

Period*	L/G†			(G-L)/O_2‡		
	Subj. C	Subj. K	Subj. N	Subj. C	Subj. K	Subj. N
I	25	32	15	17	40	78
II	66	56	44	3	25	21
III	All	All		−18	−44	

* Period I, approximately 10 P.M.–4 A.M.; Period II, approximately 4–10 A.M.; Period III, after 10 A.M.

† L/G = per cent of glucose uptake accounted for by lactate production.

‡ (G-L)/O_2 = per cent of O_2 uptake accounted for by apparent oxidation of glucose.

between arterial glucose concentration and A-V differences (Figure 2). The range of arterial glucose concentrations was 4.5 to 5.5 mM per L. or 81 to 100 mg. per 100 ml. of blood.

Spurious correlation between arterial glucose concentration and A-V difference would occur in the presence of technical error. A falsely high value for arterial glucose concentration would yield a falsely high A-V difference. Similarly, a falsely low value for venous glucose concentrate would yield a falsely high A-V difference. However, there was no correlation between venous glucose concentration and A-V difference. Since there is no reason to suspect that errors in estimate of arterial glucose were larger than those in estimate of venous glucose, it is likely that the correlation found between arterial glucose and A-V difference is not an artefact. Furthermore, even when the data are challenged by assuming

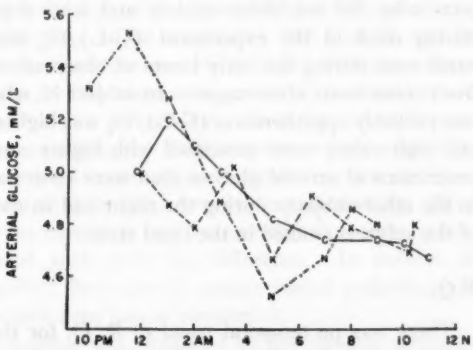


FIG. 1. DECREASE IN ARTERIAL GLUCOSE CONCENTRATION WITH TIME

C, K, and N each represent a subject.

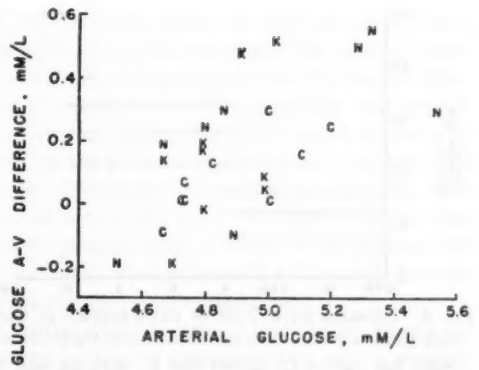


FIG. 2. CORRELATION BETWEEN ARTERIO-VENOUS DIFFERENCE AND ARTERIAL GLUCOSE CONCENTRATION

C, K, and N each represent a subject.

errors of two standard deviations in measurements of arterial glucose concentrations the correlation between arterial glucose and A-V difference is upheld.

Lactate production

Lactate was produced by resting forearm throughout the night. There was no temporal trend in lactate production. Arterial lactate concentration remained constant.

Fraction of glucose uptake accounted for by lactate production: L/G

The expression ½ (V-A lactate difference)/(A-V glucose difference) yields the fraction of glucose uptake accounted for by lactate production when concentrations are expressed in moles. This value increased during the night (Figure 3) until in Period III all the glucose uptake was accounted for by lactate production either owing to negative A-V glucose differences (three observations) or to very small A-V glucose differences in the presence of particularly large V-A lactate differences (two observations).

Fraction of O_2 uptake accounted for by oxidation of glucose: $(G-L)/O_2$

As described previously (1), when lactate production is subtracted from glucose uptake and the remaining glucose is assumed to have been oxidized an estimate can be made of the fraction of O_2 uptake accounted for by oxidation of glucose.

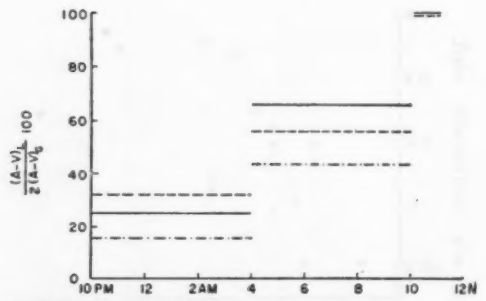


FIG. 3. INCREASE WITH TIME OF THE FRACTION OF GLUCOSE UPTAKE ACCOUNTED FOR BY LACTATE PRODUCTION

Solid line, subject C; dashed line, K; dash-dot line, N. Data represent mean values for each of three experimental periods: Period I, 10 P.M.–4 A.M.; Period II, 4 A.M.–10 A.M.; Period III, after 10 A.M., corresponding to time during which basal state observations were made previously (1).

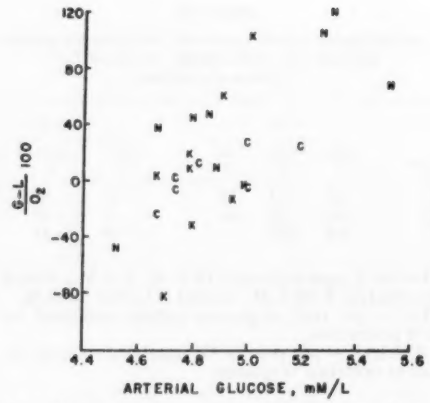


FIG. 5. CORRELATION BETWEEN (G-L)/O_2 AND ARTERIAL GLUCOSE CONCENTRATION

C, K, and N each represent a subject.

As shown in Figure 4, this fraction decreases throughout the night.

The fraction of O_2 uptake accounted for by oxidation of glucose is determined by the expression $6[(A\text{-}V)_G - \frac{1}{2}(V\text{-}A)_L]/(A\text{-}V)_{O_2}$, where subscripts refer to concentrations of glucose, lactate and oxygen, respectively. The value depends upon three A-V differences. When the fraction (G-L)/O_2 was plotted against each of these three A-V differences, in turn, it was clear that variations in the fraction were independent of A-V differences for lactate and for O_2 but were quite dependent upon variations in glucose A-V difference. Since glucose A-V difference correlated with arterial glucose concentration, it is not surprising that (G-L)/O_2 also varies with arterial glucose concentration (Figure 5). Barring the possibility, noted above and considered unlikely, that the correlation between glucose A-V difference and arterial glucose concentration is an artefact, it would appear that the higher the concentration of glucose in arterial blood, the higher the rate of glucose uptake into forearm muscle, and the greater the fraction (G-L)/O_2. The latter event occurs without demonstrable change in oxygen consumption; that is, glucose appears to be oxidized instead of rather than in addition to something else.

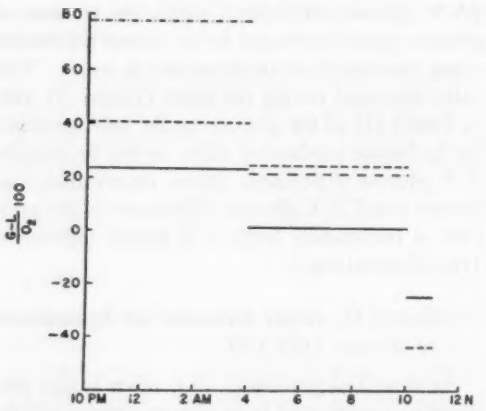


FIG. 4. DECREASE WITH TIME OF THE FRACTION OF FOREARM O_2 UPTAKE ACCOUNTED FOR BY OXIDATION OF GLUCOSE

See legend to Figure 3.

It is to be noted (Table II) that in the two subjects who did not show anxiety and who slept during most of the experiment (G-L)/O_2 was small even during the early hours of observation, five to nine hours after supper. In subject N, who was probably apprehensive, (G-L)/O_2 was higher, and high values were associated with higher concentrations of arterial glucose than were observed in the other subjects during the night and in any of the subjects studied in the basal state.

R.Q.

There was no temporal trend in R. Q. for the group as a whole. Nor was there any correlation between R.Q. and (G-L)/O_2, perhaps owing to cumulative experimental error. Mean R.Q. for

the three subjects was 0.82; for each subject it was 0.75, 0.81, and 0.89.

Hematocrit

There was no real variation in hematocrit during the experiment and there was no difference between hematocrits on paired samples of arterial and venous blood. A decrease in hematocrit with successive blood sampling, particularly noticeable between first and second sample, has been reported by others (3) but did not occur in this study. It may be noted, however, that there was some blood loss associated with insertion of the arterial needle and venous catheter, so that the sample of blood used for the first hematocrit did not represent the first blood withdrawn from these subjects.

DISCUSSION

By application of the Fick principle metabolism of resting skeletal muscle (the forearm) has been studied in three normal subjects throughout the night, approximately from 10 P. M. to 11 A. M., a period extending from four to seventeen hours after the last meal.

During this time there were fluctuations in blood flow and in O_2 consumption by the forearm, but these followed no definite temporal pattern. In fact, O_2 A-V differences were relatively constant and variations in O_2 uptake correlated with variations in blood flow in subject C. Neither forearm blood flow nor O_2 uptake was beyond the range of values obtained in a larger group of subjects examined in the basal state at least 16 hours after the last meal. It might be noted parenthetically that, on the assumption that limb O_2 uptake is constant, changes in A-V O_2 differences have been used by some as measures of changes in blood flow. The present data show that O_2 uptake is not constant and that limb blood flow cannot be estimated in this way. A similar caution has been expressed by Mottram (4) whose findings differ from those reported here in that O_2 uptake correlated with A-V O_2 difference. In subject K neither flow nor O_2 uptake varied sufficiently to permit detection of correlation.

Lactate was produced by the forearm throughout the period of observation but, despite wide variation in its production, there was again no definite temporal pattern. It has been pointed out in our previous report on subjects in the basal state that it is quite remarkable that lactate is produced under resting conditions in the face of vigorous O_2 uptake (1). The possibility was considered in that report that there might be areas of intermittent ischemia in the forearm, perhaps as a result of rhythmic vasomotor activity; we have no evidence on this point. It is clear however that lactate production is certainly not a phenomenon of starvation since it occured in the earliest measurements, four hours after a meal.

Furthermore, lactate production accounted for a large fraction of glucose uptake, on the assumption that glucose abstracted from arterial blood was the source of lactate delivered to forearm venous effluent. Since anaerobic glycolysis is less efficient, in terms of energy yield, than aerobic dissimilation of glucose, it is puzzling that the forearm should resort so extensively to lactate production when it obviously has the capacity to oxidize completely all of the glucose it has removed from arterial blood under these conditions. It might be suspected, for example, that lactate production by forearm represents muscle's contribution of carbohydrate to the rest of the body: lactate produced by muscle is delivered to liver, converted to glycogen and released ultimately as glucose. However, if lactate contributed by forearm muscles is typical quantitatively of total body muscle then the total hepatic glucose made available by this means amounts to only about 15 per cent of the glucose released by the liver, a value so small as to make it unlikely that this is an important reason for continuous lactate production by muscle. Even this small contribution represents no net gain for the body as long as muscle lactate arises from dissimilation of glucose. Another possibility is that lactate production may be useful simply because reduced coenzyme, $DPNH_2$, is oxidized during reduction of pyruvate to lactate. However, it is difficult to see that this leads to any gain since the quantity of DPN regenerated in the process is no greater than the quantity reduced during dissimilation of hexose phosphates to pyruvate. There is thus no obvious benefit derived from lactate production by resting muscle.

Glucose uptake, glucose A-V difference and arterial glucose concentration all tended to decrease with time. It will be noted in Figure 2 that there is a reasonably linear relation between ar-

terial glucose concentration and glucose A-V difference, that the A-V difference is zero at an arterial glucose concentration of approximately 4.7 mM per L., and that negative A-V differences fall on the straight line which describes the general relation. Two of the negative A-V differences differ from zero by twice the standard error of the method. Thus it would appear that negative A-V differences may have been real. Since several large series have been reported in which A-V differences in glucose concentration were either always positive or only occasionally negative and this latter not beyond experimental error (5–7) an explanation is needed.

Among several possible explanations for negative A-V glucose differences is the possibility that some of the material measured as glucose in venous blood was in fact hexose phosphate derived from skeletal muscle. Although no independent measurement of hexose phosphate was undertaken, data available in the literature (8) suggest that there is far too little hexose phosphate in venous plasma to account for the negative A-V glucose differences which were found.

Since there is no glucose-6-phosphatase in skeletal muscle, any glucose in venous effluent from muscle in excess of that in arterial supply must have diffused into venous blood from the limited mass of preformed glucose in interstitial fluid and perhaps in intracellular water. Diffusion of glucose from interstitial fluid into the blood stream will occur when glucose concentration in arterial blood falls below that in interstitial fluid. This occurs when arterial glucose concentration decreases with time and since there are no large reservoirs of extravascular glucose in muscle, such back diffusion of glucose cannot persist unless arterial glucose concentration falls progressively. When A-V glucose differences are plotted against the slope of the arterial glucose concentration it is discovered that negative A-V differences occurred at a time when arterial glucose concentration appeared to be falling, suggesting that this is the explanation for observed negative A-V differences. However, arterial samples were so infrequent that it is impossible to determine accurately the slope of the arterial glucose-time curve at any point. Since there is some uncertainty, it may be noted that negative A-V differences would occur also if the concentration of glucose in interstitial fluid were maintained at a fairly constant value. Thus, net flux of glucose from blood to interstitial fluid occurred when arterial glucose concentration exceeded 4.7 mM per L. of whole blood, or 5.7 mM per L. of water, and the net flux was reversed when arterial glucose concentration fell below this value.

Somogyi (5) has demonstrated increasing A-V glucose differences as arterial concentration rose following glucose ingestion. The observed fall in A-V difference in our subjects in association with a falling arterial glucose concentration then extends these observations and demonstrates the association over a narrow physiological range of arterial concentrations (81 to 100 mg. per cent). It must be pointed out that these alterations in rate of glucose diffusion from blood in response to small alterations in arterial glucose concentration are violations of the steady-state assumption inherent in application of the Fick principle. In the case of falling arterial concentration, apparent uptake of glucose is falsely low. However, since the changes in arterial concentration were small and probably occurred slowly, it is unlikely that the error was large in these experiments and it is quite improbable that it is large enough to alter the major conclusion that glucose uptake was very small.

Owing to the lack of trend in lactate production and in O_2 uptake together with decreasing glucose uptake, the fraction of glucose uptake accounted for by lactate production increased progressively and the fraction of O_2 uptake accounted for by glucose oxidation decreased progressively throughout the period of observation. Since decreasing glucose uptake was consequent upon decreasing arterial glucose concentration, the latter event seemed to be responsible for all the relations noted above. The decrease in arterial glucose concentration presumably represents the inability of the liver to maintain steady delivery of glucose as its glycogen stores become exhausted with time. Despite diminished availability of glucose, skeletal muscle appears to continue to produce lactate and consume O_2 at a relatively steady rate. Since it is certain that steady O_2 consumption in the face of declining glucose uptake implies a shift from glucose to other substrates within muscle, it is conceivable that steady lactate production may imply similarly that the lactate produced by muscle

may not arise entirely from glucose and that glucose may become a less important source of lactate as fasting progresses. This implies that glycogenolysis is occurring in skeletal muscle at rest. The question of the quantitative role of muscle glycogen as an oxidizable substrate in resting muscle has been treated previously (1), and the conclusion was that its role is minor.

Although it remains unknown what the major fuel for resting skeletal muscle may be, it is clear that oxidation of glucose accounts for only a minor fraction of O_2 uptake during most of the period of observation.

SUMMARY

Certain metabolic events occurring in resting skeletal muscle were studied intermittently over the hours 10 P.M. to 11 A.M., approximately, by measuring appropriate arterio-venous concentration differences and forearm blood flow in three normal men in the post-prandial state. There were patternless fluctuations in blood flow, O_2 uptake, CO_2 production and lactate production, but the range of values was generally within the variation previously found among a larger group of men who were fasted 16 hours.

Arterial glucose concentration fell progressively and this phenomenon appeared to be responsible in turn for progressive decreases in glucose A-V difference, in glucose uptake, in the fraction of O_2 uptake estimated to have been spent in oxidation of glucose, and for a progressive increase in the fraction of glucose uptake accounted for by lactate production.

During the entire period oxidation of glucose could account for only a minor fraction of O_2 uptake.

ACKNOWLEDGMENT

We are greatly indebted to Miss Ellen Rogus and Mrs. Gerda von Ahlefeldt for their assistance in the conduct of these studies and in the chemical analyses.

REFERENCES

1. Andres, R., Cader, G., and Zierler, K. L., The quantitatively minor role of carbohydrate in oxidative metabolism by skeletal muscle in intact man in the basal state. Measurements of oxygen and glucose uptake and carbon dioxide and lactate production in the forearm. J. Clin. Invest., 1956, **35**, 671.
2. Andres, R., Zierler, K. L., Anderson, H. M., Stainsby, W. N., Cader, G., Ghrayyib, A. S., and Lilienthal, J. L., Jr., Measurement of blood flow and volume in the forearm of man; with notes on the theory of indicator-dilution and on production of turbulence, hemolysis, and vasodilatation by intra-vascular injection. J. Clin. Invest., 1954, **33**, 482.
3. Hanna, C. H., and Marshall, L. H., Effect of sampling on erythrocyte, dye and protein concentrations of venous blood of dogs. Am. J. Physiol., 1955, **182**, 331.
4. Mottram, R. F., The relationship between blood flow, arterio-venous oxygen difference, and oxygen uptake of human skeletal muscle. J. Physiol., 1955, **130**, 42 P.
5. Somogyi, M., Studies of arteriovenous differences in blood sugar. I. Effect of alimentary hyperglycemia on the rate of extrahepatic glucose assimilation. J. Biol. Chem., 1948, **174**, 189.
6. Bell, D. M., Femoral arteriovenous sugar differences in fasting human beings. J. Lab. & Clin. Med., 1952, **40**, 337.
7. Elrick, H., Hlad, C. J., Jr., and Witten, T., The enhancement of peripheral glucose utilization by glucagon. J. Clin. Invest., 1955, **34**, 1830.
8. Helve, O., On blood phosphorus distribution in certain internal diseases. Acta med. Scandinav., 1946, **125**, 505.

HYPOSTHENURIA IN SICKLE CELL ANEMIA: A REVERSIBLE RENAL DEFECT

By H. G. KEITEL, D. THOMPSON, AND H. A. ITANO

(From the National Institutes of Health, Bethesda, Maryland, Freedman's Hospital, and the Department of Pediatrics, Howard University, Washington, D. C.)

(Submitted for publication March 19, 1956; accepted May 11, 1956)

The frequent occurrence of hyposthenuria in patients with sickle cell anemia has been noted since 1928 (1). McCrory, Goren, and Cornfeld (2), and Kunz, Pratt, Mellin, and Cheung (3), established that the urinary concentration defect was not due to a deficiency of the antidiuretic hormone and Zarafonetis, Steiger, Molthan, McMaster, and Colville (4), reported that subjects with the sickle cell trait also had hyposthenuria.

It has been suggested that the renal defect has a genetic basis (4); however, the data to be presented demonstrate the correction of the concentration defect in sickle cell anemia patients in whom intravascular sickling was suppressed by transfusions of normal red blood cells.

METHODS

The concentration of solutes in urine was determined cryoscopically with the Bowman freezing point apparatus (5). The accuracy attained is within two per cent. The symbol *mOsml.* used to designate the solute concentration of urine, represents that concentration which results in a freezing point depression of 1.86×10^{-3}° C. and is equivalent to a solution containing one millimole of "ideal" solute per 1,000 gm. of water.

The bisulfite method was used for determining the presence of sickle cells (6). The method of Wells and Itano was used for the identification of hemoglobin (7), and the benzidine method, as modified by Crosby, Munn, and Furth was used for the plasma hemoglobin determination (8).

Urine concentration tests

A. *The routine concentration test:* Unless otherwise indicated, the routine urine concentration test was used for the determination of the ability to concentrate urine. Ingestion of food and water was prohibited for 14 to 17 hours. The overnight urine was collected and discarded about 2 hours before the administration of Pitressin®. Two units of Pitressin® were administered subcutaneously at about the 15th hour of fasting and thirsting and another two units were administered 30 minutes later. Urine samples were collected 10, 30, and 60 minutes following the first injection of Pitressin®. The most concentrated of the three samples was considered to represent the maximal urine concentration ability under the conditions of the test. If successive urine samples showed any degree of progressive concentration, it was assumed the subject had ingested water and the test was repeated. A catheter was used to obtain urine from infants under 2 to 3 years of age and from the occasional adult who could not void. Some of the children cried following the parenteral medication; otherwise, the patients remained quiescent during the test.

In most cases the concentrations of the three urine samples were of the same order of magnitude. Occasionally the urine samples following the administration of Pitressin® were more dilute than the sample obtained before the Pitressin® was administered; this observation was confined chiefly to the infants and children who physically manifested their displeasure at having received an injection. Possibly a renal hemodynamic change or solute diuresis resulted from the crying.

B. *Simple dehydration test:* This test was identical to test A above except that Pitressin® was not used and the urine collections were extended for two one-hour periods.

C. *Urine concentration test during water administration:* The patient was urged to drink water to his maximum ability. While the patient was undergoing water diuresis and was in positive water balance amounting to about 2 to 3 per cent of body weight, Pitressin® was administered and urine collected as in the routine urine concentration test described above.

Case material

The control subjects included normal volunteers, nonsicklemic siblings of patients with sickle cell anemia and genetic dwarfs.

The sickle cell anemia patients had a history of sickle cell crises and had been hospitalized frequently. Hematological examination revealed positive sickling, predominantly "S" hemoglobin by paper electrophoresis and reticulocytosis (see Table I). Most of the older patients had received many transfusions but almost none of the patients under 3 years had been transfused. Several transfusions had been given to patients Nos. 25 and 26 within 5 months of the study. Patient No. 24 had thalassemia-sickle cell disease and patient No. 9 had sickle cell "C" disease.

RESULTS

The urine concentration test was performed on 12 white and 12 nonsicklemic negro normal con-

TABLE I

Sickle cell anemia patients

Pt.	Sex	Age *years*	Whole blood Hb *Gm. %*	Whole blood Hct. *%*	Plasma Hb	Plasma Bilirubin *mg. %*	Plasma Urea N	Retic count *%*	Urine conc. test *mOsml.*
1	F	9/12		28	2.3	0.8	9	11.5	553
2	F	1	7.8			0.3		2.6	502
3	F	1		24	11	1.7	15	21.4	682
4	M	2		24	49	2.5	9	14.7	465
5	M	2½		31	4	1.0	9	13.0	578
6	F	3	7.0			1.2		12.2	508
7	M	3	7.8			1.2			423
8	F	3	7.0			0.6			460
9	M	4		25	26	2.2	11	5.6	617
10	F	4		27	3.2	1.0			487
11	F	4		22	19	0.9	14	5.8	767
12	M	5		21	25	3.3	10	11	414
13	M	6		25	24	1.8	10	12	494
14	F	6		31	4	1.1	10	8.8	596
15	M	6		20	24	2.1	13	13.8	468
16	M	6		22	26	3.0	9	10.5	451
17	M	8	6.5			3.8			478
18	M	9	6.8						423
19	F	9		23	15	2.0	8	15.0	473
20	F	10		28	23	3.1	10	5.9	446
21	M	12		36	5	0.5	10	1.8	492
22	F	19		22	21	5.0	14	7.0	420
23	M	19	7.5		13	3.9	6	5.0	416
24	M	19		29	15	1.3	7	4.5	441
25	F	23	7.8		3.5	1.9	12	3.2	510*
26	M	23	7.7				13	5.0	502*
27	F	24		23	21	3.0	11	10.0	428
28	F	31	8.5		4	0.7	9	10.0	445
29	F	41		27		2.5	15	6.0	369

* Blood transfusions had been given within 6 months.

trols of varying ages (see Figure 1A). The average urine concentration was similar in both groups of control subjects, and in subjects of different ages. The mean value and standard deviation for the 24 control subjects was 1,055 ± 118 mOsml.

The urine concentration test was performed on 29 patients with sickle cell anemia (see Table I and Figure 1C). None of the patients was able to concentrate urine normally. No untransfused patient over 7 years of age achieved a concentration over 500 mOsml., whereas one-half of the patients under 7 years concentrated above 500 mOsml. One untransfused patient aged 4 years was able to concentrate to 767 mOsml. The blood urea nitrogen and routine urinalyses were normal in all patients.

It seemed reasonable to hypothesize that if the concentration defect were due to intravascular sickling or to sickle cell hemoglobinemia that the defect might be reversible if sickle cells were eliminated from the vascular system. Therefore multiple blood transfusions were given to seven patients. The results of urine concentration studies of four children under five years of age with sickle cell anemia who were given multiple transfusions of normal red blood cells [1] are presented in Figure 2. The maximum ability to concentrate urine increased from 553 to 682 mOsml. before the transfusions to 942 to 1042 mOsml. following the transfusions. The ability to concentrate urine returned to the pretransfusion level following cessation of transfusions and the return to the sicklemic state. As there was no decrease in the rate of excretion of solutes, the observed changes were not due to solute diuresis effect.

[1] Blood stored from one to three days, from which 80 per cent of the plasma was removed, was used for the transfusions. The details concerning the per cent sickle cells remaining in the vascular system during multiple transfusions appear elsewhere (9). Less than 1 per cent sickle cells were seen when the whole blood hemoglobin concentration was maintained at 13 to 15 gm. per cent. However, the bone marrow continues to contain a normal number of sickle cells. When the hemoglobin concentration drops below 11 gm. per cent, reticulocytes and sickle cells reappear.

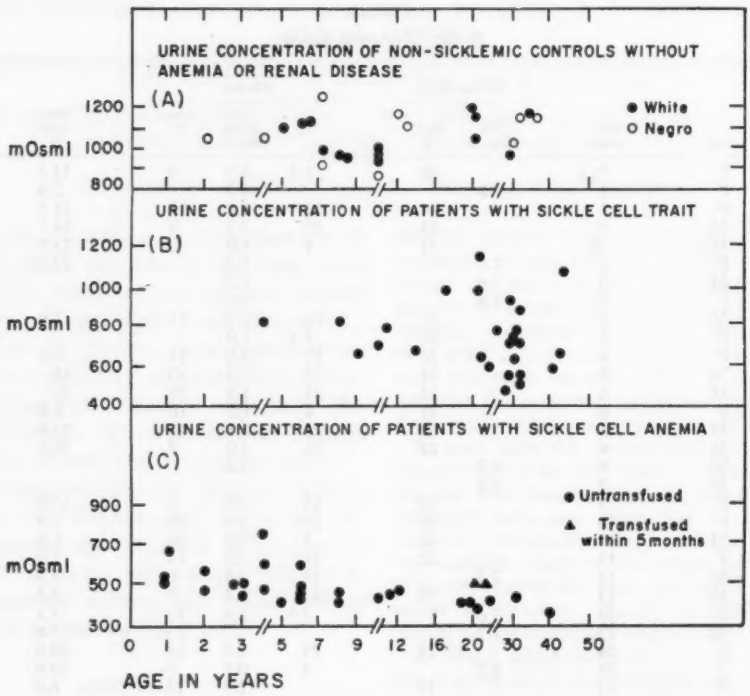


FIG. 1. THE MAXIMUM SOLUTE CONCENTRATION OF URINE AT DIFFERENT AGES IN (A), CONTROL SUBJECTS (B), SICKLE CELL TRAIT SUBJECTS, AND (C), SICKLE CELL ANEMIA PATIENTS

In a 10-year-old patient the maximum ability to concentrate urine increased from 500 mOsml. to 750 mOsml. only after 2 months of repetitive transfusions (see Figure 3). While this increase represents a substantial improvement in urine concentration, the highest concentration achieved is considerably below the lower limit attained by the control subjects. Possibly a normal maximum urine concentration would have been attained if the transfusions had been continued for a longer period. In two young adults the concentration defect was altered only slightly, or not at all, following correction of the anemia (see Figure 4). However, insufficient observations were made, and the transfusions may not have been continued for a sufficiently long period of time, to conclude that the urine concentration defect in these older patients was irreversible. The data suggest however that both the severity and reversibility of the hyposthenuria are altered with age.

A consideration of possible mechanisms resulting in hyposthenuria in sickle cell anemia

A. *The possible effect of anemia and hemoglobinemia on formation of concentrated urine.* It has been suggested that anemia, which in some instances is associated with reduced renal clearances of inulin and PAH (10), may result in hyposthenuria but this apparently is not always the case (3, 4, 11). The maximum urine specific gravity value recorded in the hospital record of patients with various types of anemia, including some with chronic anemia, is seen in Figure 5. In over one-third of the patients specific gravity values over 1.025 were observed, whereas adult patients with sickle cell anemia have urine specific gravity values that range from 1.010 to 1.020. The simple dehydration urine concentration test was performed on six adult patients with anemia not due to sickle cell disease. The results are presented in Table II. Normal urine solute concentrations were seen

in four patients and a slightly reduced value was seen in one other. The value of 685 mOsml. of patient "G", the sixth patient, is higher than those observed in all but one of the sickle cell anemia patients. The patient with homozygous "C" disease, who obviously had the disorder since birth, had a higher plasma hemoglobin concentration than many of the sickle cell anemia patients.

Sickle cell anemia patient No. 21 had been noted to have hematocrit values above 35 per cent over a one-year period; the impairment of his urinary concentration ability is as severe as in the patients with a more pronounced degree of anemia. Several sickle cell anemia patients (Nos. 1, 5, 10, 14, 21, and 28) have plasma hemoglobin concentrations which are just above the normal range (see Table I). In two of these patients (Nos. 1 and 27) it was ascertained that the plasma hemoglobin remained below 6 mg. per cent for several months, even during sickle cell crises. The urine concentration defect is as severe in these patients as in the patients with more pronounced hemoglobinemia. Since it has been shown that the degree of hemoglobinuria is usually directly related to the plasma hemoglobin concentration it would appear that hemoglobinuria also may not be the cause of the hyposthenuria.

B. *Hemodynamic factors and renal function.* A frequent finding in children with sickle cell anemia is an increase in the glomerular filtration rate (GFR) (12). However, adults with sickle cell anemia usually have a normal or even decreased GFR (13). It is not clear how an increase in the GFR could, in the absence of solute diuresis, influence the concentration of urine during hydropenia. The findings of Bruck (14) of an increase in the

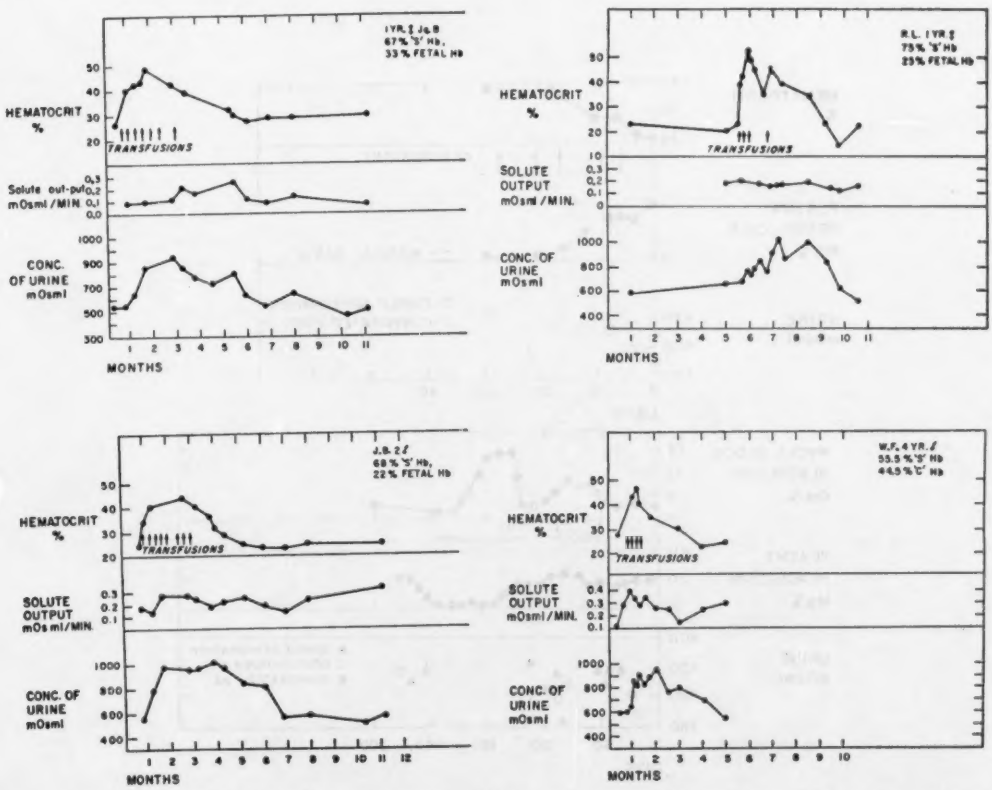


FIG. 2. THE MAXIMUM SOLUTE CONCENTRATION OF URINE IN THREE CHILDREN WITH SICKLE CELL ANEMIA, AND ONE CHILD WITH SICKLE CELL HEMOGLOBIN C DISEASE FOLLOWING MULTIPLE TRANSFUSION OF NORMAL RED BLOOD CELLS

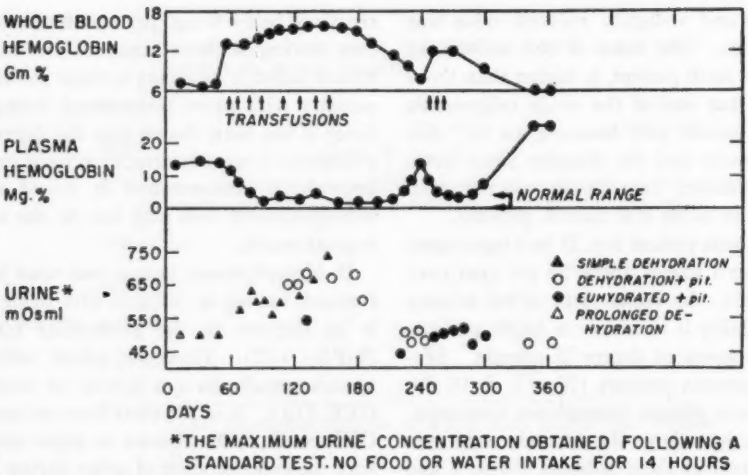


FIG. 3. THE MAXIMUM SOLUTE CONCENTRATION OF URINE IN A 10-YEAR OLD FEMALE WITH SICKLE CELL ANEMIA FOLLOWING MULTIPLE TRANSFUSIONS OF RED BLOOD CELLS

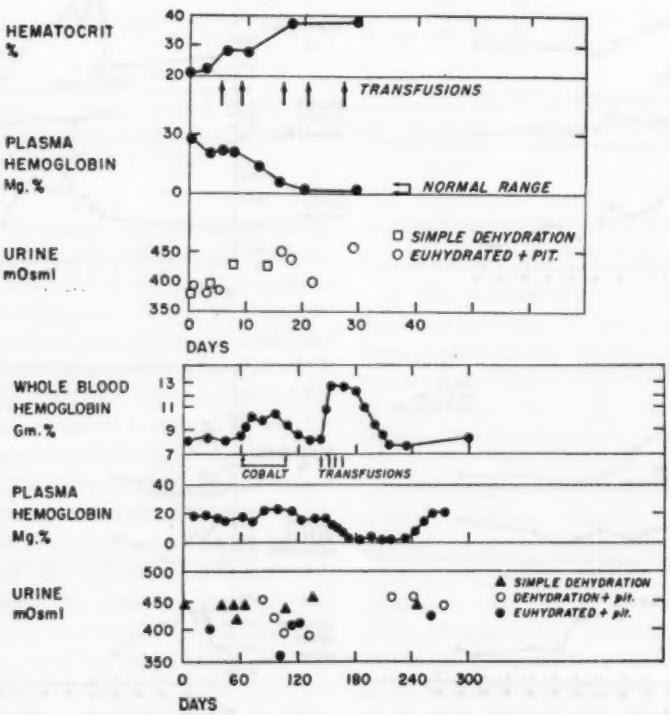


FIG. 4. THE MAXIMUM SOLUTE CONCENTRATION OF URINE IN TWO ADULTS WITH SICKLE CELL ANEMIA FOLLOWING TRANSFUSIONS OF NORMAL RED BLOOD CELLS AND COBALT MEDICATION

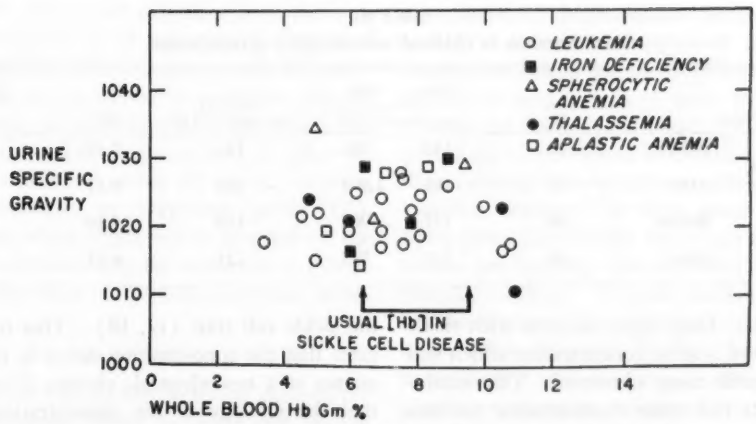


FIG. 5. THE URINE SPECIFIC GRAVITY OF MORNING FASTING URINE IN PATIENTS WITH ANEMIA NOT DUE TO SICKLE CELL DISEASE

GFR in patients with Mediterranean anemia indicate that the increase in GFR is not primarily related to the concentration defect since these patients concentrate normally (14).

Quiet standing, which resulted in syncope, and prolonged dehydration, which increased osmolality of plasma by 10 per cent, failed in each instance to alter the ability of the kidney to concentrate urine in two sickle cell anemia patients. Dehydration and quiet standing are frequently accompanied by a reduction in the GFR (15, 16).

The effect of correcting anemia on renal clearances was determined in two sickle cell anemia patients. The results which appear in Table III indicate no consistent change and confirm the observations of Bruck (14). It is clear, however, that the number of red blood cells flowing through the kidney per unit of time increased markedly following the correction of the anemia since the hematocrit was increased two-fold. However, there is little reason to believe that a disturbance in oxygenation of renal cells is related to the concentration defect. The effect of 100 per cent oxygen administration for two hours on the urine concentration of solutes was observed in two water-deprived patients with sickle cell anemia. The solute concentration of urine remained unchanged.

The urine concentration ability of subjects with the sickle cell trait

It has been suggested that the urine concentration defect in sickle cell anemia is genetic since the defect is also found in non-anemic trait parents of these patients (4). Table IV and Figure 1B indicate the results of urine concentration tests performed on 26 sickle cell trait subjects. With only two exceptions (cases Nos. 10 and 12) the subjects over 20 years of age are parents of sickle cell

TABLE II

The urine solute concentration in patients with anemia not due to sickle cell anemia

Patient	Hb. *Gm.* % and range	Urine *mOsml.*	Hb. plasma *mg.* %	Diagnosis	Duration *years*
S	9 (6–10)	957		Aplastic anemia	½
Cr	8.5 (7–10)	814		Chronic hemolytic anemia	2
G	9 (7–11)	685		Chronic hemolytic anemia	1
Sr	9 (8–10)	1,100	50–200	Chronic hemolytic anemia	1
Si	10.5	1,018	5	Thalassemia minor	39
B	7.6	863	15	Homozygous hemoglobin "C" disease	26

TABLE III

Renal clearances in sickle cell anemia: Effect of transfusions

Patient		Hct. %	GFR cc./min./1.73 M²	RPF cc./min./1.73 M²	Tm PAH mg./min./1.73 M²	F.F.	GFR / Tm PAH
V. M. 9 years Female	Before	20	148	788	124	0.19	1.2
	After	39	166	1,005	140	0.17	1.2
N. S. 19 years Male	Before	30	157	962	119	0.16	1.3
	After	40	137	785	121	0.17	1.1

disease patients. Only eight subjects with sickle cell trait achieved a urine concentration which was in the 95 percentile range of normal. The remaining 18 subjects had urine concentration maxima that are in the range described by the sickle cell anemia paients. A history of a G.U. tract infection was denied by all subjects except on No. 14. The plasma urea nitrogen concentration, routine urinalysis, plasma hemoglobin concentration and blood pressure were normal in all subjects in whom these tests were performed.

The GFR has been reported to be normal in the sickle cell trait (17, 18). This further indicates that the concentration defect is not a consequence of a hemodynamic change, if one assumes that the etiology of the concentration defect in both the trait and the sickle cell anemia patients is the same.

The effect of Pitressin® during water diuresis in sickle cell anemia and in normal controls

Two normal subjects and two patients with sickle cell anemia were given graded doses of Pitressin® intravenously during sustained water

TABLE IV

Sickle cell trait subjects

Subject	Sex	Age years	Hct. %	Plasma Hb mg. %	Plasma Bilirubin mg. %	Plasma Urea N mg. %	Retic count %	Urine conc. test mOsml.
1	M	4	39	2		15	0.6	825
2	F	8	38		0.4		0.1	831
3	M	9	*					661
4	M	10	37	1	0.3	12	0.6	703
5	M	11	37	0.8	0.2			792
6	M	14	*	0.8	0.5	15	0.3	691
7	M	17	32	1.0	0.6	13	2.2	997
8	M	22	40	0.5			1.1	927
9	F	23	36	3.5	0.5	14	1.2	641
10	F	23	43					1,113
11	M	24	46					596
12	M	26	*					784
13	F	28	44	0.5	0.7	8		470†
14	F	29	42	2.0	0.4	12		548†
15	F	29	*					937
16	F	29	39	2.3	0.7		1.2	706
17	M	31	48	2.5	0.5			774
18	M	31	*					745
19	F	31	*					623
20	M	32	47	3.1	0.4	13		712
21	F	33	37	1.0	0.5	12	1.5	519†
22	F	33	39	1.0	0.7	7	1.0	544
23	M	33	48	1.0	0.8		0.9	881
24	M	41	41	1.0	0.6	12	1.3	589†
25	F	41	30	0.5	0.3			1,091
26	M	43	*					680

* In seven subjects (3, 6, 12, 13, 15, 19, 26) whole blood hemoglobin values were reported to be normal within one year of the urine concentration study.

† Intravenous pyelogram normal.

diuresis. The Pitressin® was taken from the same lot. In all 4 subjects 0.5 milliunits of Pitressin® failed to alter the water diuresis, while 2.5 milliunits resulted in a cessation of maximum diuresis and an increase in osmolality of urine (see Table V). It would appear inappropriate to label the concentration defect in sickle cell anemia as being Pitressin®-resistant, as suggested earlier (2). This is a term which might best be reserved for the condition characterized by the resistance to Pitressin® while the kidney is forming hypotonic urine.

DISCUSSION

In face of the finding that in young patients the urine concentration defect in sickle cell anemia was completely corrected following the elimination of sicklemia, there seems to be no reasonable alternative to concluding that the urine concentration defect results from the presence of sickle cell hemoglobin. It obviously is not due to extensive renal disease since the GFR, PAH clearance (12–13), water diuresis and other renal functions (18) are often normal or supernormal. The finding that sickle cell trait subjects also exhibit the urine concentration defect indicates that the defect is not primarily due to anemia, if one assumes that the etiology of the concentration defect in these subjects is the same as in the sickle cell anemia patients. The presence of the defect in sickle cell anemia patients with only mild anemia further indicates that anemia is not primarily responsible for the renal defect.

Two possible mechanisms which might cause the defect in both the trait and anemia patients are 1) subclinical intravascular sickling in kidney and 2) a functional impairment of the concentrating mechanism due to the presence in the renal tubules or tubular cells of sickle cell hemoglobin.

Abel and Brown, and others (19–22) have reported the occurrence in sickle cell trait patients of tissue damage presumably resulting from intravascular sickling. In some cases high altitude flying precipitated sickle cell crises but usually no known predisposing circumstances favored intravascular sickling. Painless unilateral hematuria has been the most frequently reported abnormality in the sickle cell trait (19, 22). As the red cell survival time, reticulocyte count and plasma hemoglobin concentration are normal in the sickle cell trait it is quite certain that generalized intravascular hemolysis and an increased rate of red cell destruction do not usually occur (23–25). If sickle cell hemoglobin, as normal hemoglobin, is reabsorbed by the renal tubular cells from tubular urine (26–27), the presence of sickle cell hemoglobin may in some unexplained manner result in renal damage. However, the lowest concentration of "S" hemoglobin that can produce aggregation at zero oxygen tension and a pH of about 7 is between 7 and 10 Gm. per cent (28); therefore it would appear unlikely that renal intracellular precipitation of "S" hemoglobin occurs.

There is indication that severe chronic hemoglobinuria is deleterious to kidney function since only one of five of Ham's patients with chronic hemolytic anemia not due to sickle cell disease could concentrate urine normally; two of his patients had other signs of renal impairment (29). However, these patients had hemoglobinemia of

TABLE V

The effect of the intravenous administration of varying amounts of Pitressin® on the urine solute concentration and on urine flow during water diuresis in sickle cell anemia and in normal controls

Milli-units of Pitressin® given I.V.		Duration of anti-diuresis *minutes*				Pre-Pitressin® urine concentration *mOsml.*				Maximal urine concentration following Pitressin® *mOsml.*			
		25	2.5	1.5	0.5	25	2.5	1.5	0.5	25	2.5	1.5	0.5
Sickle cell anemia patients	N. S.	105	75		0	28	92		34	302	367		29
	V. M.	95	75	55	0	45	60	41	36	508	375	169	36
Normal controls*	H.	75	95	45	0	50	55	50	43	739	835	90	37
	S.	90	90	45	0	107	53	47	97	745	845	65	80

* Normal hospitalized volunteers.

much greater severity than any of our sickle cell anemia patients.

SUMMARY

1. Impairment of urine concentration was seen in 29 patients with sickle cell anemia, sickle cell "C" disease and sickle cell thalassemia. The concentration defect was not associated with other evidence of renal impairment.

2. The ability to concentrate urine was restored to normal in four infants with sickle cell disease following multiple transfusions of normal red blood cells. In a 10-year-old child the concentration defect was only slowly and incompletely corrected and in two young adults little or no change in concentration ability was observed following multiple transfusions.

3. The minimal threshold response to Pitressin® during water diuresis appears to be normal in sickle cell anemia.

4. The possibility that the urine concentration defect in sickle cell anemia and the sickle cell trait results from renal damage due to either intravascular sickling or from the presence of sickle cell hemoglobin in renal tubular cells is discussed.

5. Impairment of urine concentration was seen in 69 per cent of subjects with the sickle cell trait. In most instances the degree of impairment was less than that seen in patients with sickle cell anemia. Other evidence of renal damage was lacking.

6. Hyposthenuria is the most common complication associated with the presence of intravascular "S" hemoglobin.

ACKNOWLEDGMENTS

The authors appreciate the opportunity to thank the following people for their generous advice, support and referral of patients: Dr. R. W. Berliner, National Heart Institute, Bethesda, Maryland, Dr. Roland Scott and Dr. Melvin Jenkins, Department of Pediatrics, Howard University, Washington, D. C., Dr. W. Bullock, Department of Medicine, Howard University, Washington, D. C., Dr. W. Crosby, Walter Reed Army Medical Center, Washington, D. C., Dr. Charles Rath, Department of Medicine, Georgetown University, Washington, D. C., Miss Mollie A. Golden, nurse in the O.P.D. of the National Institutes of Health, and Dr. M. Halperin, National Institutes of Health, who provided assistance in the statistical analysis of the data.

REFERENCES

1. Josephs, H., Clinical aspects of sickle cell anemia. Bull. Johns Hopkins Hosp., 1928, **43**, 397.
2. McCrory, W. W., Goren, N., and Cornfeld, D., Demonstration of impairment of urinary concentration ability or "Pitressin-resistance" in children with sickle-cell anemia. Am. J. Dis. Child., 1953, **86**, 512.
3. Kunz, H. W., Pratt, E. L., Mellin, G. W., and Cheung, M. W., Impairment of urinary concentration in sickle cell anemia. Pediatrics, 1954, **13**, 352.
4. Zarafonetis, C. J. D., Steiger, W. A., Molthan, L., McMaster, J., and Colville, V. F., Renal defect associated with sickle cell trait and sickle cell disease. J. Lab. & Clin. Med., 1954, **44**, 959.
5. Bowman, R. L., Trantham, H. V., and Caulfield, P. A., An instrument and method for rapid, dependable determination of freezing-point depression. J. Lab. & Clin. Med., 1954, **43**, 310.
6. Daland, G. A., and Castle, W. B., A simple and rapid method for demonstrating sickling of the red blood cells: The use of reducing agents. J. Lab. & Clin. Med., 1948, **33**, 1082.
7. Wells, I. C., and Itano, H. A., Ratio of sickle-cell anemia hemoglobin to normal hemoglobin in sicklemics. J. Biol. Chem., 1951, **188**, 65.
8. Crosby, W. H., Munn, J. I., and Furth, F. W., Standardizing a method for clinical hemoglobinometry. U. S. Armed Forces Med. J., 1954, **5**, 693.
9. Chaplin, H., Keitel, H. G., and Peterson, R. E., Hematological observations on sickle cell anemia patients sustained at normal hemoglobin levels by multiple transfusions. Blood, In press.
10. Bradley, S. E., and Bradley, G. P., Renal function during chronic anemia in man. Blood, 1947, **2**, 192.
11. Hayman, J. M., Jr., Shumway, N. P., Dumke, P., and Miller, M., Experimental hyposthenuria. J. Clin. Invest., 1939, **18**, 195.
12. Etteldorf, J. N., Tuttle, A. H., and Clayton, G. W., Renal function studies in pediatrics. I. Renal hemodynamics in children with sickle cell anemia. Am. J. Dis. Child., 1952, **83**, 185.
13. Etteldorf, J. N., Smith, J. D., Tuttle, A. H., and Diggs, L. W., Renal hemodynamic studies in adults with sickle cell anemia. Am. J. Med., 1955, **18**, 243.
14. Bruck, E., Renal function in anemia. Am. J. Dis. Child., 1953, **86**, 511.
15. Brun, C., Knudsen, E. O. E., and Raaschou, F., On the cause of postsyncopal oliguria. Acta med. Scandinav., 1945, **122**, 486.
16. Young, W. F., and McCance, R. A., The secretion of urine by dehydrated and normal infants. Arch. Dis. Childhood, 1942, **17**, 65.
17. Calcagno, P. L., McLavy, J., and Kelley, T., Glomerular filtration rate in children with sickle cell disease. Pediatrics, 1950, **5**, 127.

18. Keitel, H. G., Unpublished data.
19. Abel, M. S., and Brown, C. R., Sickle cell disease with severe hematuria simulating renal neoplasm. J. A. M. A., 1948, **136**, 624.
20. Sullivan, B. H., Jr., Danger of airplane flight to persons with sicklemia. Ann. Int. Med., 1950, **32**, 338.
21. Cooley, J. C., Peterson, W. L., Engel, C. E., and Jernigan, J. P., Clinical triad of massive splenic infarction, sicklemia trait, and high altitude flying. J. A. M. A., 1954, **154**, 111.
22. Goodwin, W. E., Alston, E. F., and Semans, J. H., Hematuria and sickle cell disease: Unexplained, gross unilateral, renal hematuria in negroes, coincident with the blood sickling trait. J. Urol., 1950, **63**, 79.
23. Singer, K., Robin, S., King, J. C., and Jefferson, R. N., The life span of the sickle cell and the pathogenesis of sickle cell anemia. J. Lab. & Clin. Med., 1948, **33**, 975.
24. Callender, S. T. E., Nickel, J. F., Moore, C. V., and Powell, E. O., Sickle cell disease: Studied by measuring the survival of transfused red blood cells. J. Lab. & Clin. Med., 1949, **34**, 90.
25. Neel, J. V., The inheritance of the sickling phenomenon, with particular reference to sickle cell disease. Blood, 1951, **6**, 389.
26. Oliver, J., The Edward Gamaliel Janeway Lecture. The structure of the metabolic process in the nephron. J. Mt. Sinai Hosp., 1948, **15**, 175.
27. Finch, C. A., Hegsted, M., Kinney, T. D., Thomas, E. D., Rath, C. E., Haskins, D., Finch, S., and Fluharty, R. G., Iron metabolism. The pathophysiology of iron storage. Blood, 1950, **5**, 983.
28. Singer, K., and Singer, L., Studies on abnormal hemoglobins. VIII. The gelling phenomenon of sickle cell hemoglobin: its biologic and diagnostic significance. Blood, 1953, **8**, 1008.
29. Ham, T. H., Studies on destruction of red blood cells. I. Chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria: An investigation of the mechanism of hemolysis, with observations on five cases. Arch. Int. Med., 1939, **64**, 1271.

THE DIFFUSING CAPACITY OF THE LUNGS IN PATIENTS WITH MITRAL STENOSIS STUDIED POST-OPERATIVELY [1]

By R. L. RILEY, C. J. JOHNS,[2] G. COHEN, J. E. COHN, D. G. CARROLL, AND R. H. SHEPARD [3]

(From the Departments of Environmental Medicine and of Medicine, The Johns Hopkins University and Hospital, Baltimore, Md.)

(Submitted for publication November 2, 1955; accepted May 24, 1956)

The fact that morphological changes occur in the lungs of patients with mitral stenosis has been generally accepted since 1936 when Parker and Weiss described thickening of the capillary basement membrane, scarring and probable loss of capillaries (1). These findings have been confirmed and extended by others (2, 3), yet there is no agreement regarding the physiological and clinical significance of the pulmonary lesions. Because of their location in relation to vascular structures, one would expect that such lesions might interfere with the flow of blood through the lungs or with the diffusion of gases across the pulmonary membrane. Hemodynamic studies by a number of different workers have demonstrated an increase in pulmonary vascular resistance in patients with mitral stenosis (4–10). This paper deals with studies of the diffusion of gases across the pulmonary membrane in a group of patients on whom mitral valvulotomy had been performed.

The diffusing capacity for oxygen is the number of ml. of oxygen diffusing across the pulmonary membrane per minute in response to a mean difference of 1 mm. Hg in the partial pressure of oxygen on the two sides of the membrane. It is a function of the thickness, physicochemical structure and area of the surface across which diffusion takes place. The area is related to the area of the walls of all capillaries which are actively involved in gas exchange. Capillaries are not included if they are not perfused with blood and do not take part in gas exchange. This qualification is of great importance since recent studies suggest that at rest only a fraction of the total capillary bed is perfused (11). With increase in cardiac output in response to exercise the diffusing capacity approaches a maximal value, probably because an increasing fraction of the total capillary bed takes part in gas exchange.

In an earlier study in this laboratory low diffusing capacities were demonstrated pre-operatively in some patients with mitral stenosis studied at rest (12). It was impossible to decide whether these low values indicated morphological changes in the diffusing surface or simply a smaller than usual fraction of perfused capillaries. The present study was designed to minimize this difficulty by dealing only with patients who could tolerate the combined stress of exercise and hypoxia, a combination which is effective in producing maximal values for diffusing capacity in normal persons. The finding of diffusing capacities during exercise which approach the predicted normal values for maximal diffusing capacity would provide evidence against the presence of extensive structural lesions involving the pulmonary membrane. Low results might be equivocal because of uncertainties regarding the fraction of the total capillary bed perfused, but very low values would suggest the presence of extensive structural changes.

CLINICAL MATERIAL AND METHODS

The subjects were 14 patients who had had incapacitating mitral stenosis of long standing but who had improved sufficiently following mitral valvulotomy to tolerate the combined stress of exercise and hypoxia. They were believed to have little or no mitral insufficiency on the basis of the clinical examination and the surgeon's impression at operation. Vital statistics and clinical information are listed in Table I. The pre-operative studies performed at rest on 5 of these patients (M. C., F. G.,

[1] This study was aided by a contract between the Office of Naval Research, Department of the Navy, and The Johns Hopkins University (NR 112-101) and by funds provided under contract AF 18 (600)-435 with the USAF School of Aviation Medicine, Randolph Field, Texas.

[2] Fellow of the National Foundation for Infantile Paralysis.

[3] Medical Research Fellow of the National Tuberculosis Association. (Administered by the National Research Council, July 1953 to July 1955.)

TABLE I

Physical characteristics of patients and pre-operative clinical data

Subject	Sex	Age	Ht. (*cm.*)	BSA* (*sq. m.*)	Years since onset of ARF†	Dyspnea	Failure	Digitalis	Hemoptysis	Fibrillation
N. R.	F	46	163	1.74	31	2	1	1½	2	1
F. G.	F	25	162	1.65	11	3	6/12	2	1/12	6/12
D. E.	M	31	187	1.99	28	3	None	None	2	1/12
J. W.	F	38	160	1.45	None	4	2	4	None	None
W. L.	M	37	178	1.98	31	8	None	3	7	None
D. B.	F	40	175	1.89	30	5	2/12	None	2/12	None
E. S.	F	39	160	1.58	None	5	1/12	1/12	7/12	None
J. A.	M	24	183	2.07	12	3	1	1	9/12	8
G. B.	M	24	183	1.83	None	4	None	None	4	None
M. C.	F	22	157	1.47	6	11/12	None	11/12	2/12	None
T. P.	M	39	170	1.80	30	2	None	11/12	2	None
L. E.	F	35	155	1.47	23	3	None	3	6	None
M. P.	F	27	151	1.34	18	2	1	10/12	None	None
R. D.	F	39	159	1.48	None	7	2	3	None	1

* BSA: body surface area.
† ARF: acute rheumatic fever.

J. A., L. E., and R. D.) were part of the previous report (12). Similar studies at rest have been reported by others (5, 13, 14).

Studies of ventilation, of the distribution of blood and gas within the lungs and of diffusing capacity were performed. Vital capacity and maximal breathing capacity measurements were compared to the normal standards of Baldwin, Cournand, and Richards, as re-analyzed by Armstrong (15, 16). The distribution of blood and gas within the lungs was evaluated by means of the ratio of "dead space" to tidal volume and the ratio of "venous admixture" to total blood flow, according to a system of analysis which has been reported (17–19). The upper limit of normal for the ratio of "dead space" to tidal volume is considered to be .30 and for the ratio of "venous admixture" to total flow, .06.

Diffusing capacity was determined by the method of Lilienthal, Riley, Proemmel, and Franke (20). The patients' exercise tolerance was not adequate to permit determinations at several different levels of work as can be done with normal subjects (11). Work was performed on a motor-driven treadmill at rates which on clinical grounds seemed as strenuous as the patients could safely tolerate at the lower level of oxygenation. The duration of exercise was 6 to 10 minutes. Every patient except one (R. D.) consumed oxygen at a rate in excess of 400 ml. per min. per sq. meter body surface, and all had diffusion gradients at the end of the alveolar capillaries ($P_A^* - P_c^*$) of 6 mm. Hg or more. In every instance the arterial blood oxygen tension was 50 mm. Hg or below at the lower level of oxygenation.

The determinations of "dead space," "venous admixture" and diffusing capacity required simultaneous sampling of expired gas and arterial blood at two different levels of oxygenation. A value for mixed venous blood is also necessary, and simultaneous sampling of mixed venous blood would have been desirable theoretically but might have been dangerous during treadmill exercise. Values for mixed venous blood at either extreme of the physiologic range were therefore assumed and a corresponding range for diffusing capacity calculated. The assumed values for oxygen saturation of mixed venous blood were such as to produce differences in saturation between the blood leaving the pulmonary capillaries and the mixed venous blood (S_c^*–$\bar{S}v$) of 40 and 70 per cent during exercise and 25 and 50 per cent at rest. Since the saturation of the blood leaving the capillaries (S_c^*) was approximately 80 per cent at the lower level of oxygenation, the assumed mixed venous blood values ($\bar{S}v$) were approximately 40 and 10 per cent, respectively, for the studies during exercise. They were approximately 55 and 30 per cent for the studies at rest. The range of values for diffusing capacity yielded by this procedure is sufficiently precise for the problems under consideration.

The values obtained for diffusing capacity were compared to the maximal values reported by Cohn, Carroll, Armstrong, Shepard, and Riley for normal men of different ages (21). Since adequate normal standards for women are not available, three-fourths of the value for a man of the same age and height was used in the case of female subjects. This procedure is consistent with the findings in the 9 normal women in the same age group whose maximal diffusing capacities have been determined in this laboratory to date. If the predicted normal value in any instance is taken to be 100 per cent, a given determination will be within one standard deviation if it falls within 19 per cent of the predicted normal, *e.g.*, between 81 and 119 per cent.

RESULTS

In all of the tables the patients are arranged in order of decreasing diffusing capacity during exercise. The data of patients whose highest diffusing capacity during exercise was within 1 standard de-

TABLE II

Ventilatory capacities and values related to blood-gas distribution in the lungs

Subject	Months post-op.	Vital capacity		Maximal breathing capacity		% Dead space $V_D^*/V_T^* \times 100$	Range of % venous admixture $Q_{va}/Q_t \times 100$†
		L.	*% N.**	*L. min.*	*% N.**		
N. R.	41	2.58	96	67	82	30	3.5–2
F. G.	26	3.38	108	106	112	18	2–1
D. E.	55	5.70	129	155	114	26	3–2
J. W.	6	3.75	133	89	102	26	1–0.5
	21	3.84	136	107	120	24	2.5–1.5
W. L.	24	5.95	145	177	136	26	3–1.5
	41	4.49	116	126	116	26	4–2.5
D. B.	37	3.73	116	132	136	30	4.5–3
E. S.	29	2.37	87	88	105	28	4–2.5
	42	2.20	81	62	75	28	5–3
J. A.	4	4.32	95	128	87	22	3–1.5
	31	4.38	97	129	98	24	7.5–5
G. B.	25	4.45	99	117	87	32	1.5–1
M. C.	5	2.11	68			19	2.5–1.5
T. P.	5	3.67	93	148	127	22	1–0.5
	9	3.88	99	132	113	26	4–2.5
	33	4.08	108	147	134	35	4–2.5
L. E.	29	2.39	88	62	75	30	2–1
M. P.	11	2.39	83	87	100	39	3–1.5
R. D.	11	2.12	74	87	88	31	7–4
	38	2.12	78	76	92	39	14–9

* Predicted normal values for vital capacity and maximal breathing capacity decrease with age and therefore, in a given patient, the predicted normal values change when the studies are separated by a period of many months.

† Values for % venous admixture are dependent upon the composition of the mixed venous blood and hence upon the assumed values for $S_a^* - \bar{S}v$ (see text).

viation of the predicted normal maximal value appear above the horizontal line; the lower values are below the line. No helpful correlation between the pre-operative clinical information and the diffusing capacity during exercise is evident (Table I), and abnormalities in ventilatory capacities and in values related to blood-gas distribution in the lungs are not significantly correlated with abnormalities in diffusing capacity (Table II).

Diffusing capacities and related values are presented in Table III. Three of the 5 patients for whom serial data are available show increases in diffusing capacity during exercise over periods of 4, 15 and 27 months, but these changes are not beyond one standard deviation (19 per cent of the predicted normal maximal value). In a fourth patient (W. L.) a significant decrease in diffusing capacity occurred between 24 and 41 months post-operatively. In Figure 1 the range of diffusing capacity is shown for all post-operative studies during exercise. These data are plotted as percentage of the predicted normal maximal values and are shown in relation to the number of months after operation. Approximately half of the patients had diffusing capacities during exercise which were within 1 standard deviation of the predicted normal maximal values.

In the 6 patients for whom pre-operative studies at rest are reported, the diffusing capacity at rest was 24 per cent or less of the predicted normal maximal value. Normal standards for diffusing capacity at rest have not been defined clearly for the reason that the normal alveolar-capillary diffusion gradient at the end of the capillary ($P_A^* - P_c^*$

TABLE III

Diffusing capacity and related values †

Subject	Months post-op.	$\frac{\dot{V}_{O_2}}{M^2}$	Pa_{O_2}	Range of $P_A^* - P_c^*$	Range of $P_A^* - \bar{P}c$	Assumed range $S_c^* - \bar{S}v$	Pred. max. D_{O_2}	Range of observed D_{O_2}	Range of % pred. max. D_{O_2}
N. R.	41	488	43	11–10	21–27	40–70	31	40–31	129–100
F. G.	−½*	178*	32*	27*	30–34*	25–50*	40	9–8*	22–20*
	26	530	36	10	18–22	40–70	39	49–40	126–102
D. E.	55	762	43	10	20–26	40–70	65	76–58	117– 89
J. W.	6	422	50	7	20–26	40–70	34	31–24	91– 71
	21	510	34	14	21–26	40–70	33	35–29	106– 88
W. L.	24	470	43	6	16–20	40–70	56	59–47	105– 84
	41	470	31	20	26–31	40–70	55	36–30	65– 56
D. B.	6*	117*	47*	9*	14–19*	25–50*	40	16–11*	40– 27*
	37	470	44	11	22–28	40–70	39	40–32	103– 82
E. S.	29	464	46	11	23–30	40–70	32	32–25	100– 78
	42	424	45	10–11	22–29	40–70	31	30–23	97– 74
J. A.	−2½*	149*	47*	15–14*	27–32*	25–50*	68	11– 9*	16– 13*
	−1*	197*	38*	20*	27–31*	25–50*	68	15–13*	22– 19*
	4	442	40	8	17–21	40–70	67	51–42	76– 63
	31	728	33	17	24–30	40–70	66	65–52	98– 78
G. B.	25	675	41	12	21–27	40–70	67	59–46	88– 69
M. C.	−1*	147*	46*	19–18*	29–34*	25–50*	39	7– 6*	18– 15*
	5	498	43	14	25–31	40–70	39	29–24	74– 61
T. P.	5	470	45	20	32–38	40–70	50	26–22	52– 44
	9	556	45	14	26–32	40–70	50	38–31	76– 62
	33	586	40	20–19	30–35	40–70	49	35–30	71– 61
L. E.	−½*	128*	39*	16–15*	23–27*	25–50*	33	8– 7*	24– 21*
	29	403	37	19	28–33	40–70	32	22–18	69– 56
M. P.	−1*	99*	40*	23*	29–34*	25–50*	34	5– 4*	15– 12*
	11	403	37	19	31–36	40–70	34	18–16	53– 47
	21*	108*	38*	7*	12–16*	25–50*	33	12– 9*	36– 27*
R. D.	−1*	151*	41*	46–47*	63–68*	25–50*	34	3*	9*
	−½*	135*	42*	17–16*	25–30*	25–50*	34	8– 6*	24– 18*
	1*	115*	48*	18–12*	30–32*	25–50*	34	6– 5*	18– 15*
	2*	147*	46*	20–18*	32–36*	25–50*	34	6*	18*
	11*	107*	41*	13–12*	20–24*	25–50*	33	8– 7*	24– 21*
	11	238	41	16	27–33	40–70	33	13–11	39– 33
	38	360	42	26	40–47	40–70	31	13–11	42– 35

* Indicates data obtained at rest.

† $\dot{V}_{O_2}/M^2$ = O_2 consumption in ml. per minute S.T.P.D. (Standard temperature and pressure, dry) per square meter of body surface area.

Pa_{O_2} = arterial P_{O_2} in mm. Hg.

$P_A^* - \bar{P}c$ = Mean alveolo-capillary oxygen tension gradient in mm. Hg, or "effective" alveolar-mean capillary oxygen tension gradient.

$S_c^* - \bar{S}v$ = Difference in oxygen saturation between blood leaving the alveolar capillaries and mixed venous blood, or "effective" capillary-mixed venous difference in oxygen saturation.

Max. D_{O_2} = Maximal diffusing capacity for oxygen.

$$\text{Per cent of predicted normal maximal } D_{O_2} = \frac{\text{Observed } D_{O_2} \times 100}{\text{Predicted normal max. } D_{O_2}}.$$

in Table III) is often less than 6 mm. Hg and hence too small to determine without a large percentile error. In the 6 patients studied preoperatively at rest, $P_A^* - P_c^*$ ranged between 14 and 47 mm. Hg. In view of these large gradients, there can be little doubt that the corresponding val-

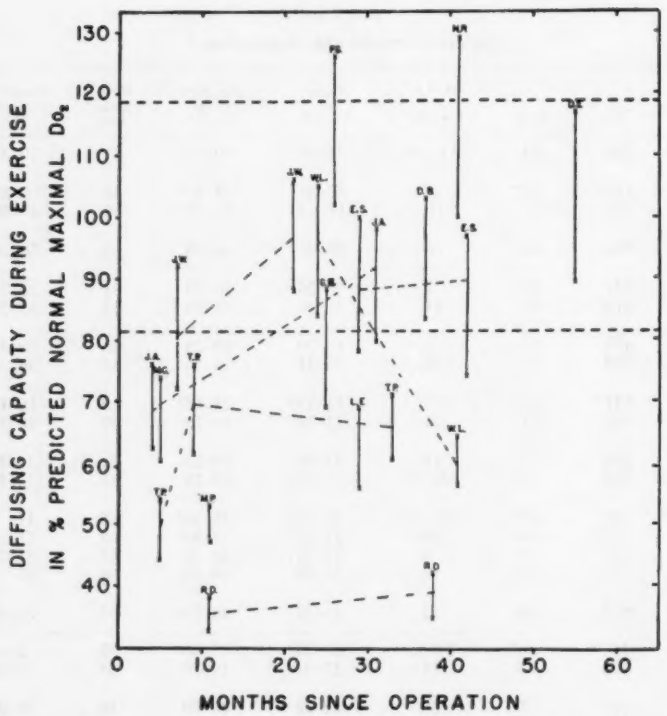


FIG. 1. DIFFUSING CAPACITY DURING EXERCISE IS PLOTTED AGAINST THE NUMBER OF MONTHS SINCE OPERATION

The vertical lines indicate the range within which each diffusing capacity value falls, based on calculations using an extreme range of assumed values for mixed venous blood (see text). The results of successive studies on the same patient are connected by broken lines. The horizontal broken lines enclose a range of one standard deviation above and below the predicted normal value (100 per cent). Note that the ordinate scale does not begin at zero.

ues for diffusing capacity at rest were abnormally low.[4]

When the patients who were studied pre-operatively at rest had additional studies post-operatively during exercise, there was a striking increase in diffusing capacity in every case. There was no correlation between the values obtained pre-operatively at rest and those obtained post-operatively during exercise. Clinically all patients were markedly improved after operation and had greatly increased exercise tolerance.[5]

DISCUSSION

The patients whose diffusing capacities approached the maximal values for normal people must have been both close to normal and close to maximal with respect to diffusion. The fact that they were close to normal indicates that extensive lesions of the pulmonary membrane probably were not present at the time of study. The fact that they were close to maximal indicates that the determination of the maximal, or nearly maximal, diffusing capacity is technically feasible in at least some patients who have had mitral stenosis.

[4] Furthermore, by the criterion used in a previous publication (12) all these determinations at rest would be considered low.

[5] R. D. died suddenly at home 4½ years after valvulotomy. The x-ray picture of the chest had for several years shown multiple nodular densities indicating structural changes in the lungs which may have been related to the rheumatic process.

This is of particular interest because the level of exercise which the patients performed was less than that which is required of normal people in obtaining maximal values for diffusing capacity, and the patients' cardiac output values were probably considerably below those of normal persons during the determination of the maximal diffusing capacity. These considerations suggest that the increased pulmonary capillary pressure and/or other hemodynamic alterations in the patients with mitral stenosis tend to increase the proportion of pulmonary capillaries perfused at a given level of cardiac output.

The diffusing capacities which were below normal during exercise might have been low because of structural changes in the diffusing surface or because many of the pulmonary capillaries were not perfused with blood under the conditions of the study. A choice between these alternatives is difficult to make in the case of the moderately low values, but the very low values obtained in patients M. P. and R. D. probably reflect extensive structural damage to the diffusing surface.

Although the factors causing the low pre-operative diffusing capacities at rest cannot all be evaluated, the subsequent post-operative finding of considerably higher values during exercise suggests that some of the factors were reversible. On theoretical grounds it seems possible that the low preoperative diffusing capacities might have been related to low cardiac output, high critical closing pressure of small pulmonary vessels (12), or subclinical pulmonary edema—all potentially reversible.

With respect to the selection of patients for surgery, the experience with this small group indicates that striking clinical improvement from valvulotomy may be obtained even in patients with severe impairment of diffusion. Normal people have a large reserve of diffusing capacity which is required only during very heavy exercise. The limitation of exercise tolerance in patients with mitral stenosis reduces the need for this reserve. Impairment of diffusion therefore appears to be of secondary importance in this group of patients.

SUMMARY

1. Studies of pulmonary function are reported in 14 patients on whom mitral valvulotomy was performed 5 to 55 months previously. Pulmonary ventilation, intrapulmonary distribution of blood and gas, and diffusing capacity were measured. Findings were related to pre-operative studies previously reported.

2. Although significant incapacity had existed over a period of years pre-operatively, approximately half of the patients showed post-operative diffusing capacities during exercise which were within one standard deviation of the predicted normal values for maximal diffusing capacity. The other patients showed moderate to severe lowering of diffusing capacity during exercise.

3. The patients with diffusing capacities during exercise which were normal probably did not have extensive structural changes in the pulmonary membrane. The 2 with very low values probably did have extensive structural changes. Intermediate values could not be interpreted.

4. All 6 of the patients who were studied pre-operatively at rest showed low diffusing capacities. Since all were greatly improved following mitral valvulotomy, a low pre-operative diffusing capacity is not considered a contraindication to surgery.

ACKNOWLEDGMENT

The authors wish to acknowledge the continued interest of Dr. E. Cowles Andrus, who made possible most of the long term follow-up studies.

REFERENCES

1. Parker, F., Jr., and Weiss, S., The nature and significance of the structural changes in the lungs in mitral stenosis. Am. J. Path., 1936, **12**, 573.
2. Larrabee, W. F., Parker, R. L., and Edwards, J. E., Pathology of intrapulmonary arteries and arterioles in mitral stenosis. Proc. Staff Meet., Mayo Clin., 1952, **24**, 316.
3. Henry, E. W., The small pulmonary vessels in mitral stenosis. Brit. Heart J., 1952, **14**, 406.
4. Welch, K. J., Johnson, J., and Zinsser, H., The significance of pulmonary vascular lesions in the selection of patients for mitral valve surgery. Ann. Surg., 1950, **132**, 1027.
5. Curti, P. C., Cohen, G., Castleman, B., Scannell, J. G., Friedlich, A. L., and Myers, G. S., Respiratory and circulatory studies of patients with mitral stenosis. Circulation, 1953, **8**, 893.
6. Dexter, L., Pathologic physiology of mitral stenosis and its surgical implications. Bull. New York Acad. Med., 1952, **28**, 90.

7. Draper, A., Heimbecker, R., Daley, R., Carroll, D., Mudd, G., Wells, R., Falholt, W., Andrus, E. C., and Bing, R. J., Physiologic studies in mitral valvular disease. Circulation, 1951, **3**, 531.
8. Werkö, L., Biörck, G., Crafoord, C., Wulff, H., Krook, H., and Eliasch, H., Pulmonary circulatory dynamics in mitral stenosis before and after commissurotomy. Am. Heart J., 1953, **45**, 477.
9. Ferrer, M. I., Harvey, R. M., Wylie, R. H., Himmelstein, A., Lambert, A., Kuschner, M., Cournand, A., and Richards, D. W., Circulatory effects of mitral commissurotomy with particular reference to selection of patients for surgery. Circulation, 1955, **12**, 7.
10. Judson, W. E., Hatcher, J. D., Hollander, W., and Halperin, M. H., The effects of mitral valvuloplasty on cardiovascular and renal function at rest and during exercise. J. Clin. Invest., 1955, **34**, 1297.
11. Riley, R. L., Shepard, R. H., Cohn, J. E., Carroll, D. G., and Armstrong, B. W., Maximal diffusing capacity of the lungs. J. Applied Physiol., 1954, **6**, 573.
12. Carroll, D., Cohn, J. E., and Riley, R. L., Pulmonary function in mitral valvular disease: Distribution and diffusion characteristics in resting patients. J. Clin. Invest., 1953, **32**, 510.
13. Blount, S. G., Jr., McCord, M. C., and Anderson, L. L., The alveolar-arterial oxygen pressure gradient in mitral stenosis. J. Clin. Invest., 1952, **31**, 840.
14. Williams, M. H., Jr., Pulmonary function studies in mitral stenosis before and after commissurotomy. J. Clin. Invest., 1953, **32**, 1094.
15. Baldwin, E. deF., Cournand, A., and Richards, D. W., Jr., Pulmonary insufficiency. I: Physiological classification, clinical methods of analysis, standard values in normal subjects. Medicine, 1948, **27**, 243.
16. Armstrong, B. W., Unpublished line charts.
17. Riley, R. L., and Cournand, A., "Ideal" alveolar air and the analysis of ventilation perfusion relationships in the lungs. J. Applied Physiol., 1949, **1**, 825.
18. Riley, R. L., and Cournand, A., Analysis of factors affecting partial pressures of oxygen and carbon dioxide in gas and blood of lungs: Theory. J. Applied Physiol., 1951, **4**, 77.
19. Riley, R. L., Cournand, A., and Donald, K. W., Analysis of factors affecting partial pressures of oxygen and carbon dioxide in gas and blood of lungs: Methods. J. Applied Physiol., 1951, **4**, 102.
20. Lilienthal, J. L., Jr., Riley, R. L., Proemmel, D. D., and Franke, R. E., An experimental analysis in man of the oxygen pressure gradient from alveolar air to arterial blood during rest and exercise at sea level and at altitude. Am. J. Physiol., 1946, **147**, 199.
21. Cohn, J. E., Carroll, D. G., Armstrong, B. W., Shepard, R. H., and Riley, R. L., Maximal diffusing capacity of the lung in normal male subjects of different ages. J. Applied Physiol., 1954, **6**, 588.

AN EVALUATION OF POTASSIUM IODIDE AS A THERAPEUTIC AGENT IN THE TREATMENT OF EXPERIMENTAL HYPERCHOLESTEREMIA AND ATHEROSCLEROSIS [1]

By MEYER FRIEDMAN, RICHARD HOMER,[2] and SANFORD O. BYERS with the technical assistance of CLARENCE OMOTO and WARREN HAYASHI

(From the Harold Brunn Institute for Cardiovascular Research, Mount Zion Hospital, San Francisco 15, Calif.)

(Submitted for publication March 19, 1956; accepted May 24, 1956)

Although iodides have been administered for many decades to patients suffering from arteriosclerotic disorders, it was Liebig (1, 2) who first furnished experimental data possibly justifying the use of this substance in such disorders. He observed that the majority of his cholesterol fed rabbits could be protected against subsequent aortic atherosclerosis if iodide were administered.

Since his initial observations, various other studies have been done concerning the possible protective effect of iodide administration against both experimentally induced hypercholesteremia and atherosclerosis. Some (3–6) have agreed with the conclusions of Liebig but others (7, 8) were unable to demonstrate any specific anti-hypercholesteremic or anti-atherogenic effect of iodide administration. Furthermore, although Turner (3) initially observed that iodide administration was protective, he and Bidwell (9, 10) later observed that at best the supposed protection by iodide administration was but a temporary phenomenon. Finally, Brown and Page (11) were unable to detect any effect of iodide administration upon the hepatic cholesterol content of the normal, cholesterol fed rabbit.

In view of these sometimes conflicting reports it was decided therefore to pursue a series of studies concerning the possible effects of iodide administration both upon normo- and hypercholesteremic animals with the intent of determining the actual efficacy of this cation both as an antihypercholesteremic and antiatherogenic agent. The results clearly indicate that iodide *per se* possesses neither of these two effects.

[1] Aided by Grants from the Life Insurance Medical Research Fund and the National Institutes of Health, Grant H-119 (C6), Public Health Service.

[2] Work done while Public Health Service Research Fellow, National Heart Institute, National Institutes of Health, HF-4788.

I. *The Effect of Potassium Iodide Upon Intestinal Absorption of Cholesterol*

Methods

A. *Rats:* A series of male Long-Evans rats (average weight: 235 to 251 grams) previously starved for 24 hours was given 25 mg. of cholesterol in 1.0 ml. of olive oil by stomach tube and then divided into four groups. Group I (17 rats) which served as a control received only the 25 mg. of cholesterol. Group II (9 rats) received in addition 25 mg. of KI with the cholesterol. Group III (8 rats) received 100 mg. of KI in addition to the cholesterol. Group IV (7 rats) received 200 mg. of KI plus the cholesterol. All of the animals then were anesthetized with ether, the abdomen incised, the intestinal lymph duct cannulated and the lymph collected for 24 hours by methods previously described (12). This collected lymph then was analyzed for total cholesterol by the method of Saifer and Kammerer (13) and for total lipid by the method of Bragdon (14).

B. *Rabbits:* A series of young male rabbits (average weight: 1,424 to 1,436 grams) was given 800 mg. of cholesterol dissolved in 8 ml. of olive oil by stomach tube and then divided into two groups. Group I (16 rabbits) which served as a control received only the cholesterol. Group II (10 rabbits) received in addition to the cholesterol, 1,250 mg. of KI. The animals then were caged individually and fed a cholesterol-free diet (leafy vegetables) for 72 hours. All feces excreted during this period were individually collected and analyzed for total sterol, total cholesterol, and non-cholesterol sterol according to methods previously described (12).

Results

Inspection of Tables I and II will indicate that the oral administration of potassium iodide had no significant effect upon the intestinal absorption of cholesterol in either the rat or the rabbit. Regardless of whether the rat was given 25, 100 or 200 mg. of KI, the total amount of cholesterol absorbed during the 24 hours following the ingestion of 25 mg. of cholesterol did not appear to differ from that observed in the control animals (*cf.* A with B, C and D of Table I). Similarly, no

TABLE I

The effect of KI on absorption of cholesterol and total lipid in the rat

No. of rats		Average weight gm.	Lymph Volume ml.	Lymph Total cholesterol mg./100 ml.	Lymph Total cholesterol mg./24 hrs.	Lymph Total lipid mg./100 ml.	Lymph Total lipid mg./24 hrs.
		A. Control rats given 25 mg. of cholesterol					
17		248	29.6	66	18.6	1,974	560
	Range:	(225–300)	(21.5–43.5)	(38–106)	(13.0–28.0)	(1,230–3,290)	(320–990)
	S.E. Mean:				±0.9		±37
		B. Rats given 25 mg. of cholesterol + 25 mg. of KI					
9		241	31.7	58	17.6	1,948	514
	Range:	(234–255)	(23–45.5)	(44–92)	(12.0–24)	(1,000–2,960)	(270–930)
	S.E. Mean:				±1.3		±65
		C. Rats given 25 mg. of cholesterol + 100 mg. of KI					
8		235	41.0	52	20.6	2,043	793
	Range:	(210–278)	(29–52)	(37–61)	(17.5–24.8)	(1,315–3,950)	(510–1,190)
	S.E. Mean:				±0.9		±91
		D. Rats given 25 mg. of cholesterol + 200 mg. of KI					
7		251	29.8	57	17.1	1,783	529
	Range:	(226–274)	(23–38)	(46–71)	(14.0–22.0)	(1,543–2,300)	(365–600)
	S.E. Mean:				±1.3		±35

effect of KI administration upon the absorption of total lipid was observed.

Similarly in the rabbits, whether one employs the total sterol or the total cholesterol contents of the 72-hour intestinal excretion as an indicator, no significant difference was found in the excretion of cholesterol in the rabbit fed KI in addition to the 800 mg. of cholesterol. This similarity in the excretion of total sterol and cholesterol in the two groups of course implies that a similarity in the amount of ingested cholesterol absorbed must also have existed.

II. *The Effect of Potassium Iodide Upon Intestinal Excretion of Cholesterol*

Methods

This function was stiudied in the rat. A group of 10 male Long-Evans rats (average weight: 233 to 248 grams) was given 3 ml. of olive oil and then half of the rats were given 200 mg. of KI. The animals then were individually caged, given a sterol-free diet and the feces were collected for 72 hours and analyzed for total sterol, total cholesterol and non-cholesterol sterol as done above.

Results

The ingestion of KI did not appear to influence the intestinal excretion of cholesterol. Thus (see Table III) the total sterol and cholesterol excretion of the control rats was 29.8 and 15.1 mg., respectively, as compared to 32.3 and 12.9 mg., respectively, in the rats given KI. Calculation of the Standard Error of the Difference of Means indicates good agreement between these sets of values.

III. *The Acute and Chronic Effect of Potassium Iodide Upon Plasma Cholesterol and Total Lipid*

Methods

A. *Rats:* The acute effect of KI upon the plasma cholesterol and total lipid of the rats was studied by giving one series of rats a stock diet of Purina laboratory chow and a second series, the same diet enriched with cholesterol (one per cent). Both series were given 200 mg. of KI by stomach tube every other day for seven days. Two additional series of rats were given the same two diets without KI administration for control purposes. At the end of seven days, the animals were sacrificed and the liver was obtained. Plasma samples obtained at the beginning and end of the experiment were analyzed for cholesterol and total lipid. The liver was analyzed for cholesterol.

The chronic effect of KI was studied by administering 200 mg. of KI every other day for 21 days to one of two series of rats ingesting Purina chow enriched with cholesterol (two per cent) and cholate (two per cent). Plasma samples obtained at the beginning and end of

TABLE II

The effect of KI on absorption of cholesterol in the rabbit

No. of rabbits		Average weight gm.	Intestinal excretion—72 hours: Dry weight gm.	Total sterol mg./72 hrs.	Total cholesterol mg./72 hrs.	Non-cholesterol sterol mg./72 hrs.
		Control rabbits given 800 mg. of cholesterol				
16		1,436	35.9	463	352	110
	Range:	(1,190–1,749)	(20.0–47.9)	(125–777)	(110–577)	(28–309)
	S.E. Mean:			±51	±36	±25
		Rabbits given 800 mg. of cholesterol + 1,250 mg. of KI				
10		1,424	35.1	475	363	105
	Range:	(1,310–1,645)	(23.6–47.0)	(196–833)	(150–603)	(27–230)
	S.E. Mean:			±58	±48	±19

the experiment were analyzed for cholesterol and total lipids.

B. *Rabbits:* The acute effect of KI upon plasma cholesterol and total lipid of the rabbit was studied by giving in one dose, 1250 mg. of KI by stomach tube to half of a group of 20 rabbits that also were given 800 mg. of cholesterol in 8 ml. of olive oil. Plasma samples obtained before and 72 hours after the administration of the cholesterol were analyzed for plasma cholesterol.

The chronic effect of KI was studied by placing 20 rabbits upon a high cholesterol diet consisting of ground stock rabbit pellets together with added cholesterol (0.5 per cent) and potassium iodide (0.25 per cent). A control series of 20 rabbits was given the same diet except potassium chloride was substituted for the iodide. It was calculated that these rabbits ingested approximately 300 mg. of cholesterol and 150 mg. of KI per day during the 21 days of feeding. Plasma samples obtained before and seven, 14 and 21 days after the beginning of the diet were analyzed for cholesterol and total lipid.

Results

A. *Rats:* Whether rats were ingesting a stock diet or the cholesterol enriched diet, the administration of KI in the dosage employed appeared to effect acutely a slight but apparently a significant rise in the plasma cholesterol at the end of the seven-day period (see Table IV, Sections I and II). This hypercholesteremic effect of KI has been reported previously (15). The plasma lipid concentration however did not appear to be affected by the administration of KI.

The administration of KI for 21 days however to rats ingesting an hypercholesteremia-inducing diet appeared to have little or no effect upon either their plasma cholesterol or total lipid (see Table IV, Section III).

B. *Rabbits:* The administration of a single dose of 1250 mg. of KI did not appear to have any effect upon the acute hypercholesteremia occurring in rabbits given a single dose of 800 mg. of cholesterol. The average plasma cholesterol of the 10 rabbits given the single dose of cholesterol and iodide was 78 before and 252 mg. per 100 ml.

TABLE III

The effect of KI on excretion of cholesterol in the rat

No. of rats		Average weight gm.	Intestinal excretion—72 hours: Dry weight gm.	Total sterols mg./72 hrs.	Total cholesterol mg./72 hrs.	Non-cholesterol sterol mg./72 hrs.
		Control rats given 3 ml. of olive oil				
5		233	2.3	29.8	15.1	14.7
	Range:	(228–246)	(1.8–2.8)	(23.1–34.0)	(9.5–19.5)	(12.0–21.0)
	S.E. Mean:			±1.7	±1.7	±1.4
		Rats given 3 ml. of olive oil + 200 mg. of KI				
5		248	2.0	32.3	12.9	19.4
	Range:	(232–266)	(1.6–2.9)	(21.4–47.6)	(9.7–16.4)	(12.0–31.2)
	S.E. Mean:			±4.1	±1.2	±3.0

(S.E. Mean ± 19) 72 hours later. Comparable values obtained in the 10 rabbits given only the cholesterol were 81 and 286 mg. per 100 ml. (S.E. Mean ± 36), respectively.

The chronic administration of KI to rabbits was found to diminish the food intake of nine of the 20 rabbits and in such rabbits, a slight fall in weight occurred. A similar lessened food intake and fall in weight occurred in only three of the 20 control rabbits receiving KCl in their cholesterol enriched diet. For purposes of comparison, therefore, cholesterol and lipid values obtained from the rabbits who ate well and gained weight in each series were tabulated separately from those who failed to do so.

As Table V demonstrates, those rabbits ingesting KI in their high colesterol diet who both ate well and gained weight, exhibited as great an increase in their cholesterol and total lipid content as was observed in the control rabbits that gained weight while ingesting KCl. On the other hand, far less hypercholesteremia and hyperlipemia were observed in the nine rabbits fed iodide and the three control rabbits fed chloride that ate poorly and lost weight in the three-week period. (See Table V, Sections I-B and II-B.)

IV. *The Effect of Potassium Iodide Upon Biliary Excretion of Cholesterol*

Methods

Ten male Long-Evans rats (average weight: 194 grams) were placed upon a sterol free diet for 72 hours. In addition, five of these rats were given 200 mg. of KI in one ml. of H_2O by stomach tube daily. Immediately following the last administration of KI, all of the rats then were anesthetized with ether, the abdomen incised and the bile duct cannulated and bile was collected for 24 hours. The bile samples were analyzed for total cholesterol. The methods employed both for the cannulation and the cholesterol analysis of the bile have been previously described (16).

Results

The volume of bile as well as its total cholesterol content was essentially the same in both the iodide treated and control animals. The average volume of bile excreted was 14.5 ml. per 24 hrs. (Range: 11.0 to 16.8 ml.) in the iodide treated and 14.6 ml. per 24 hrs. (Range: 13.0 to 16.3 ml.) in the control animals. The average total cholesterol excreted in 24 hours was 1.42 mg. (Range: 1.07 to 1.58) in the former and 1.35 mg. (Range: 0.97 to 1.61) in the latter group of animals. This observed failure of iodide to alter the biliary excretion of cholesterol suggests that no change in the hepatic rate of synthesis of cholesterol (17) is mediated by iodide ingestion.

V. *The Effect of Potassium Iodide Upon Various Endogenous Types of Hypercholesteremia*

A. *Hypercholesteremia Induced by Intravenous Injection of Hypercholesteremic Serum*

Methods

Two series of male Long-Evans rats were placed upon a sterol free diet for 72 hours during which time they were given 200 mg. of KI daily by stomach tube. Immediately after the last iodide feeding, the first series (15 rats) was injected intravenously with 20 mg. of cholesterol in the form of pooled hypercholesteremic serum (3 ml.) previously obtained from cholate fed, bile obstructed rats (18). The second series (10 rats) was given 20 mg. of cholesterol in the form of pooled hypercholesteremic serum (2.5 ml.) previously obtained from cholesterol fed rabbits. Two control series of rats also on the sterol free diet but receiving no iodide were also injected with the two types of sera, respectively. Plasma samples obtained prior to and then immediately, six, 12 and 24 hours after the injection of the hypercholesteremic sera were analyzed for cholesterol.

Results

As previously observed, administration of iodide (prior to injection of rat hypercholesteremic serum) alone elevated the average plasma cholesterol of the first series from 49 (S.E. Mean ± 2.1) to 92 mg. per 100 ml. (S.E. Mean ± 5.4) whereas the control series exhibited an average plasma cholesterol of 42 (S.E. Mean ± 2.8) before and 65 mg. per 100 ml. (S. E. Mean ± 3.7) 72 hours after being placed upon the sterol free diet. When the rat hypercholesteremic serum was given to the experimental and control series, both groups became immediately hypercholesteremic but the pre-injection divergence in values was maintained (see Figure 1). The rate of disappearance of the excess plasma cholesterol however proceeded at approximately the same rate (see Figure 1) in the iodide and control series, respectively. A similar train of events was observed in the iodide treated and control series given rabbit hypercholesteremic serum (see Figure 1).

TABLE IV

The acute and chronic effect of KI on plasma cholesterol, lipid and hepatic cholesterol of the rat given various diets

No. of rats		Average weight gm.	Plasma cholesterol (mg./100 ml.)			Plasma lipid (mg./100 ml.)			Liver (after 7 days)		
			Before	7 days	21 days	Before	7 days	21 days	Dry weight	Cholesterol mg./100 g.	Cholesterol total mg./organ
					I. Stock diet						
					A. Control rats						
17		225	65	59		242	200		2.99	795	23.6
	Range:	(188–256)	(46–80)	(45–71)		(162–309)	(117–279)		(2.1–3.9)	(653–910)	(18.4–30.9)
	S.E. Mean:		±2.6	±2.3						±42	±0.8
					B. Rats given 200 mg. of KI every other day						
17		216	62	72		264	185		2.58	950	24.8
	Range:	(198–224)	(51–82)	(57–102)		(197–338)	(133–276)		(2.1–3.3)	(692–1,720)	(15.3–45.0)
	S.E. Mean:		±2.1	±2.3						±60	±1.7
					II. Stock diet + 1% cholesterol						
					A. Control rats						
17		212	52	59		204	212		3.07	1,407	43.1
	Range:	(196–230)	(30–66)	(40–74)		(136–273)	(173–291)		(2.3–3.8)	(950–2,530)	(28.0–81.6)
	S.E. Mean:		±2.3	±2.1						±106	±3.5
					B. Rats given 200 mg. of KI every other day						
14		219	59	79		205	206		2.62	1,483	38.4
	Range:	(194–248)	(46–76)	(60–126)		(130–409)	(119–409)		(1.8–3.5)	(846–2,730)	(19.9–63.5)
	S.E. Mean:		±2.4	±4.3						±130	±3.3
					III. Stock diet + 2% cholesterol + 2% cholate						
					A. Control rats						
14		291	58	131	120	243		259			
	Range:	(242–290)	(47–67)	(99–202)	(52–187)	(124–187)		(204–339)			
	S.E. Mean:		±2.4	±7.0	±11	±23		±10			
					B. Rats given 200 mg. of KI every other day						
12		243	59	150	124	250		290			
	Range:	(212–290)	(48–69)	(91–262)	(85–165)	(159–327)		(207–361)			
	S.E. Mean:		±2.1	±17	±8	±14		±15			

TABLE V

The chronic effect of KI on plasma cholesterol and lipid of rabbits on high cholesterol diet

No. of rabbits		Average weight (*gm.*)		Plasma cholesterol (*mg./100 ml.*)				Plasma total lipid (*mg./100 ml.*)	
		Before	21 days	Before	7 days	14 days	21 days	Before	21 days
				I. Control rabbits given KCl					
				A. Rabbits gaining weight					
17		1,898	2,066	58	184	281	284	204	553
	Range:	(1,685–2,665)	(1,760–2,885)	(46–74)	(92–327)	(67–474)	(81–984)	(149–363)	(145–188)
	S.E. Mean:			±2.1	±25	±76	±62	±17	±119
				B. Rabbits losing weight					
3		1,697	1,577	54			97	170	303
	Range:	(1,610–1,795)	(1,500–1,710)	(48–59)			(79–120)	(61–281)	(140–428)
				II. Rabbits given KI					
				A. Rabbits gaining weight					
11		1,964	2,024	63	215	271	340	236	680
	Range:	(1,765–2,190)	(1,810–2,365)	(49–87)	(114–417)	(194–715)	(120–943)	(154–356)	(264–1,935)
	S.E. Mean:			±2.1	±36	±73	±71	±25	±160
				B. Rabbits losing weight					
9		1,846	1,728	62	118	119	149	217	397
	Range:	(1,590–2,105)	(1,670–1,990)	(32–91)	(77–168)	(85–156)	(65–402)	(88–307)	(187–918)
	S.E. Mean:			±2.3	±24	±25	±32	±21	±84

The results of both these experiments left little doubt that iodide administration while elevating the average plasma cholesterol slightly did not alter the rate of disappearance of injected cholesterol when it was administered in physiological form.

B. *Hypercholesteremia Induced by Biliary Obstruction and by Injection of Triton*

Methods

Thirty-two male Long-Evans rats (average weight: 246 grams) were subjected to ligation of their biliary duct immediately after 15 of the rats were given 200 mg. of KI by stomach tube. Plasma samples obtained before and 24 hours after the ligation were analyzed for total cholesterol and total lipid.

A series of 20 male Long-Evans rats (average weight: 274 grams) were injected intravenously with 100 mg. of Triton WR-1339 [3] after half of the animals were given 200 mg. of KI by stomach tube. Plasma samples obtained before and 24 hours after the injection were analyzed for total cholesterol and total lipid.

Results

The hypercholesteremia and hyperlipemia resulting after biliary obstruction was not altered by the acute administration of KI (see Table VI).

Similarly, the hypercholesteremic and hyperlipemic effect of injected Triton was not altered by acute administration of KI (see Table VI).

VI. *The Effect of Potassium Iodide Upon the Atherosclerosis of the Cholesterol Fed Rabbit*

Methods

Two series of feeding experiments were carried out successively. In the first series, 10 of a group of 20 male albino rabbits (average weight: approximately 2,000 grams) were placed upon a diet consisting of ground rabbit pellets containing 3.5 mg. of cholesterol dissolved in 0.1 ml. of olive oil, plus 1.75 mg. of KI, per gram of pellet mixture. The remaining 10 rabbits serving as controls received the identical mixture except that KCl was substituted for KI. The daily food consumption of each group of rabbits was measured daily and the weights of the animals were obtained every three weeks. Blood samples obtained prior to, and then three, six, nine, 12 and 15 weeks after institution of the special cholesterolcation diet, were analyzed for total cholesterol and total lipids. At the end of 15 weeks, the animals were sacrificed and their aortas were examined and graded (0 to 4) for the presence of atherosclerosis.

The second series of 20 rabbits was begun after the termination of the study of the first series. Because a marked difference in food consumption between the two groups of the first series had been observed the two groups of rabbits of the second series were pair fed although each group received one of the two cholesterolcation enriched diets described above. The weights of the animals were obtained monthly. Blood samples obtained prior to and then four, 12 and 16 weeks after institution of the diet were analyzed for total cholesterol and lipid. The animals were sacrificed at 16 weeks and besides gross estimation of the degree of aortic atherosclerosis, a segment of the entire aorta (consisting of the first 5 cm. of its length from the aortic cusps) was obtained and analyzed for total cholesterol content.

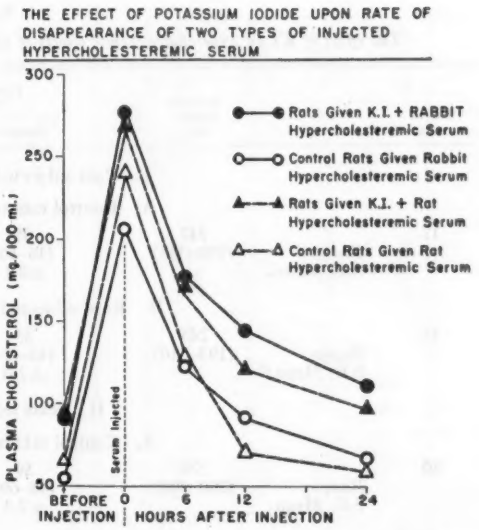


FIG. 1. THE EFFECT OF POTASSIUM IODIDE UPON RATE OF DISAPPEARANCE OF TWO TYPES OF INJECTED HYPERCHOLESTEREMIC SERUM

Results

First series. Nine of the rabbits fed KI and six of the rabbits fed KCl survived the experimental period of 15 weeks. The death of the remainder appeared to be due to cardiac tamponade following blood sampling. It was observed that although both groups of rabbits gained about equally during the 15-week period, the rabbits fed iodide approximately three weeks after being on the diet, ingested 20 to 40 per cent less of the food than did the animals fed KCl, again exhibiting the de-

[3] A para-isooctyl poloxyethylene phenol polymeric detergent kindly furnished by Rohm and Haas Co., Philadelphia, Penna.

TABLE VI

The effect of KI on hypercholesteremic effects of (a) biliary obstruction and (b) Triton injection in the rat

No. of rats		Average weight gm.	Plasma cholesterol (mg./100 ml.) Before	Plasma cholesterol (mg./100 ml.) 24 hours	Plasma lipid (mg./100 ml.) Before	Plasma lipid (mg./100 ml.) 24 hours
		I. Rats subjected to ligation of bile duct				
		A. Control rats subjected to ligation only				
17		247	55	140	181	336
	Range:	(190–306)	(46–56)	(100–184)	(163–185)	(228–470)
	S.E. Mean:		±2.1	±5.9	±7.7	±47.0
		B. Rats subjected to ligation and given KI				
15		245	55	152	210	342
	Range:	(193–310)	(44–66)	(108–200)	(206–214)	(188–412)
	S.E. Mean:		±2.0	±6.9	±2.0	±46.0
		II. Rats injected with Triton				
		A. Control rats injected with Triton only				
10		278	59	415	191	2,695
	Range:	(200–304)	(48–69)	(336–489)	(112–302)	(1,638–3,880)
	S.E. Mean:		±2.3	±12	±18	±187
		B. Rats injected with Triton and given KI				
10		269	56	362	172	2,615
	Range:	(198–308)	(42–85)	(204–477)	(103–302)	(1,853–4,960)
	S.E. Mean:		±2.4	±24	±55	±870

layed anoretic propensity of iodide ingestion previously noted. Thus, whereas the average daily consumption of the two groups was 93 and 94 grams per day, respectively, during the first three weeks, the average daily ingestion of the rabbits fed KCl increased to 218 grams per day from the third to the 15th week whereas the ingestion of the rabbits fed KI increased only to 158 grams per day. This difference in ingestion of course meant that the rabbits fed KCl were obtaining an average of 763 mg. of cholesterol and 382 mg. of KCl per day as compared to the 553 mg. of cholesterol and 276 mg. of KI per day ingested by the rabbits fed iodide. Despite this difference in intake, however, there was little difference in the average weights of the two groups at the end of 15 weeks. Thus the initial average weights of the KI and KCl groups were 1,792 and 1,789 grams, respectively, and at the end of the 15 weeks, their average weights were 3,344 and 3,419 grams, respectively. In other words, the difference in food intake of the two groups could not have been detected by mere measurement of the body weight of the two groups.

Although the average plasma cholesterol of the rabbits fed KI increased during the feeding period from 60 (Range: 31 to 86) to 1,048 mg. per 100 ml. (Range: 635 to 1,500) at the end of the 15-week period, the plasma cholesterol after the third week was consistently less than that observed in the rabbits fed KCl. It should be observed nevertheless that at the end of the feeding period, the average plasma cholesterol of the rabbits fed KI had reached a marked hypercholesteremic level. The average plasma cholesterol of the group fed KCl increased from 55 (Range: 37 to 72) to 1,327 mg. per 100 ml. (Range: 1,010 to 1,525) during the same period. The plasma total lipid content of the two groups similarly differed. Thus the average plasma lipid of the rabbits fed KI was 223, 421, 393, 513, 891 and 1,362 mg. per 100 ml. at the beginning and after three, six, nine, 12, and 15 weeks, respectively, of the feeding program. The average plasma lipid of the rabbits fed KCl was 182, 398, 248, 874, 1,219, and 1,622 mg. per 100 ml., respectively, at similar periods.

The divergency in plasma cholesterol and total lipid between the two groups was reflected in the incidence and degree of atherosclerosis found in the aorta. Thus five of the nine rabbits fed KI exhibited no gross atherosclerosis whereas each of the six rabbits fed KCl had some aortic atherosclerotic infiltration. Judged on the scale of one

to four, the average degree of atherosclerosis was 1.2 (Range: 0 to 3.0) in the rabbits fed KI and 2.5 (Range: 1 to 4) in the rabbits fed KCl.

Second series. As Table VII demonstrates, when the intake of food and cholesterol was kept exactly the same in the rabbits fed KI and KCl the increases in the average plasma cholesterol and total lipid were approximately the same in the two series during the entire feeding period.

The findings in the aorta also were essentially similar in the two series. On gross inspection, eight of the 10 rabbits fed KI and six of the eight rabbits fed KCl exhibited aortic atherosclerosis. The average degree of deposition was judged to be 1.7 (Range: 0 to 4.5) in the rabbits fed KI and 1.8 (Range: 0 to 4.0) in the rabbits fed KCl. The average cholesterol content of the aortas of the two groups also was approximately the same being 1,528 mg. per 100 grams of aorta in the rabbits fed KI and 1,730 in the animals fed KCl. Calculation of the Standard Error of the Difference of Means indicates that a good agreement exists between the two values. When sections of aortas of each group were stained with Sudan IV and examined microscopically, no difference could be observed in either the intimal hyperplasia or the deposition of lipid in the aortas of either group.

TABLE VII

The effect of KI on atherosclerosis occurring in cholesterol fed rabbits

No. of rabbits		Average weight		Average plasma cholesterol (*mg./100 ml.*)			Plasma lipid (*mg./100 ml.*)			Aorta	
		Beginning	16 weeks	Beginning	8 weeks	12 weeks	16 weeks	Beginning	8 weeks	12 weeks	16 weeks	Atherosclerosis (1–5)	Cholesterol content *mg./100 gm.*
						A. Rabbits getting KI plus cholesterol							
10	Range:	2,269 (2,090–2,520)	3,096 (2,800–3,687)	40 (21–71)	324 (109–517)	687 (415–1,100)	876 (414–1,020)	166 (136–228)	588 (414–945)	1,313 (820–1,775)	1,706 (1,165–2,490)	1.7 (0.0–4.5)	1,528 (900–2,623)
	S.E. Mean:				±39	±51	±36	±10	±50	±83	±137		±180
						B. Rabbits getting KCl plus cholesterol							
8	Range:	2,405 (2,170–2,595)	3,209 (2,683–3,553)	47 (33–64)	337 (75–906)	866 (202–1,490)	805 (232–1,020)	292 (128–570)	462 (140–1,220)	1,073 (315–1,783)	1,735 (401–2,880)	1.8 (0.0–4.0)	1,730 (875–2,525)
	S.E. Mean:				±104	±130	±82	±60	±144	±166	±256		±252
						C. Control rabbits on stock diet							
3	Range:	2,337 (2,250–2,595)	3,192 (3,065–3,261)	66 (62–70)		69 (50–70)		184 (128–240)			200 (190–220)	0	>714 (661–776)

The divergency observed in the degree of hy-

DISCUSSION

Except for a moderate acute and temporary hypercholesteremia (15), the administration of potassium iodide was not found in these studies to have any significant, specific effect upon the intestinal absorption or excretion of cholesterol nor upon its endogenous disposition, including the plasma level of cholesterol.

The results obtained in our first series of rabbits given KI plus cholesterol in their diet appeared to indicate that unless special measures were taken, such rabbits became initially anoretic, hence ingested less cholesterol and as a consequence exhibited a lower plasma cholesterol and less atherosclerosis than the control animals. However when the control rabbits of the second series were not allowed to ingest any more food and cholesterol than that taken up by the rabbits fed iodide, no differences in plasma cholesterol and atherosclerosis were observed between the rabbits fed iodide and those given chloride.

percholesteremia, hyperlipemia and aortic atherosclerosis between our two series appears to us to furnish a probable explanation why so many earlier investigators believed iodide was an antihypercholesteremic and anti-atherogenic agent. Their studies were done upon rabbits ingesting cholesterol-containing food at will and seemingly iodide administration depresses such food intake markedly after a few weeks. Since the appetite of the control rabbits was not similarly affected, the degree of hypercholesteremia and consequent atherosclerosis obviously became more marked in the latter at least during the first 15 weeks of feeding. However the cholesterol level in the blood of even the first series of rabbits fed KI was becoming high enough to be compatible with the eventual deposition of cholesterol in the aorta. This last observation perhaps explains the later observation of Turner and Bidwell (9) that the effect of iodide administration in rabbits fed cholesterol was lost after a few months.

SUMMARY

In a study employing both the rat and rabbit, the administration of iodide was not found to alter the intestinal absorption or excretion of cholesterol. Further, except for a slight initial hypercholesteremic effect in the rat, iodide administration was not found to alter either the normal or abnormal disposition of endogenous cholesterol. Similarly it was not found to alter the hypercholesteremic response of the rat to cholesterol enriched food or that of the rabbit (if allowance were made in this animal for the delayed anoretic effect of iodide administration).

Rabbits given iodide and a high cholesterol diet exhibited as much aortic atherosclerosis and cholesterol deposition as control rabbits when special measures were taken to ensure equal intake of a cholesterol enriched diet.

REFERENCES

1. Liebig, H., Experimentelle Untersuchungen über die Jodwirkung auf die Atherosklerose. Med. Klin., 1929, **25**, 1100.
2. Liebig, H., Die Beeinflussung der experimentellen Atherosklerose durch Jodbehandlung. Arch. f. exper. Path. u. Pharmakol., 1931, **159**, 265.
3. Turner, K. B., Studies on the prevention of cholesterol atherosclerosis in rabbits. I. The effects of whole thyroid and potassium iodide. J. Exper. Med., 1933, **58**, 115.
4. Turner, K. B., and Khayat, G. B., Studies on the prevention of cholesterol atherosclerosis in rabbits. II. The influence of thyroidectomy upon the protective action of potassium iodide. J. Exper. Med., 1933, **58**, 127.
5. Ungar, H., Zur Wirkung des Jods auf die Cholesterin-Atheromatose der Kaninchen. Arch. f. exper. Path. u. Pharmakol., 1934, **175**, 536.
6. Breusch, F., and Thiersch, H., Der Einfluss des Jods auf die Kaninchenatheromatose. Ztschr. f. d. ges. exper. Med., 1935, **95**, 458.
7. Rosenthal, S. R., Studies in atherosclerosis: Chemical, experimental and morphologic. V. Possible dangers of iodine therapy in atherosclerosis of aorta seen from an experimental standpoint. Arch. Path., 1934, **18**, 827.
8. Page, I. H., and Bernhard, W. G., Cholesterol-induced atherosclerosis. Its prevention in rabbits by the feeding of an organic iodine compound. Arch. Path., 1935, **19**, 530.
9. Turner, K. B., and Bidwell, E. H., Further observations on the blood cholesterol of rabbits in relation to atherosclerosis. J. Exper. Med., 1935, **62**, 721.
10. Turner, K. B., and Bidwell, E. H., Some effects of iodine given to rabbits after a brief period of cholesterol feeding. Proc. Soc. Exper. Biol. & Med., 1937, **35**, 656.
11. Brown, H. B., and Page, I. H., The effect of oral iodide on serum butanol-insoluble protein-bound iodine in various species. Circulation, 1954, **10**, 714.
12. Byers, S. O., Friedman, M., and Gunning, B., Observations concerning the production and excretion of cholesterol in mammals. XI. The intestinal site of absorption and excretion of cholesterol. Am. J. Physiol., 1953, **175**, 375.
13. Saifer, A., and Kammerer, O. F., Photometric determination of total cholesterol in plasma or serum by a modified Liebermann-Burchard reaction. J. Biol. Chem., 1946, **164**, 657.
14. Bragdon, J. H., Colorimetric determination of blood lipides. J. Biol. Chem., 1951, **190**, 513.
15. Homer, R. S., Effect of potassium iodide on plasma cholesterol of rats. Proc. Soc. Exper. Biol. & Med., 1955, **88**, 354.
16. Friedman, M., Byers, S. O., and Michaelis, F., Observations concerning production and excretion of cholesterol in mammals. II. Excretion of bile in the rat. Am. J. Physiol., 1950, **162**, 575.
17. Byers, S. O., and Friedman, M., Production and excretion of cholesterol in mammals. VII. Biliary cholesterol; increment and indicator of hepatic synthesis of cholesterol. Am. J. Physiol., 1952, **168**, 297.
18. Friedman, M., Byers, S. O., and Gunning, B., Observations concerning production and excretion of cholesterol in mammals. VIII. Fate of injected cholesterol in the animal body. Am. J. Physiol., 1953, **172**, 309.

THE METABOLISM OF CHYLOMICRA. I. THE REMOVAL OF PALMITIC ACID–1–C^{14} LABELED CHYLOMICRA FROM DOG PLASMA

By RICHARD J. HAVEL[1] AND DONALD S. FREDRICKSON

(From the Section on Metabolism, Laboratory of Cellular Physiology and Metabolism, National Heart Institute, National Institutes of Health, Bethesda, Md.)

(Submitted for publication April 23, 1956; accepted May 24, 1956)

It is now generally accepted that the intestinal lymphatics constitute the exclusive pathway for the absorption of long chain fatty acids, whether ingested unesterified or as glycerides. These fatty acids appear in the thoracic duct lymph as chylomicra. The subsequent fate of the absorbed fat has been explored very little, and the role of plasma lipoproteins in chylomicron transport and the sites of removal of the absorbed fat remain unknown.

Approximately 95 per cent of long chain fatty acids appear in the lymph as triglycerides and 5 per cent as phospholipids (1). Bergström, Borgström, and Rottenberg (2) fed labeled stearic acid and tristearin to fasting rats. Over a period of 3 to 4 hours, approximately 1 mg. per minute of fatty acids was delivered to and removed from the total plasma, which contained only about 5 mg. of preformed triglycerides. The maximum specific activities of plasma triglyceride fatty acids were only 4 and 15 per cent of the specific activities of the fed stearic acid and tristearin, respectively. The authors suggested that the absorbed triglycerides were not mixing appreciably with the existing plasma triglycerides. Their data did not exclude the possibility that the plasma triglycerides were exchanging rapidly with a large triglyceride pool.

Jones, Gofman, Lindgren, Lyon, Graham, Strisower, and Nichols (3) studied the low-density lipoproteins following fat feeding in humans and found a transient elevation of lipoproteins having flotation rates greater than S_f 60, followed by a stepwise degradation to higher density molecules with flotation rates down to, but not below, S_f 30. They suggested that the low-density lipoproteins constitute a transport system for absorbed fatty acids.

In most studies of exogenous fat metabolism, interpretation of removal rates and mechanisms has been hampered by the variable and uncontrollable factor of fat absorption. In a few studies, chyle has been injected intravenously in order to obviate this difficulty. Marble, Field, Drinker, and Smith (4) found that intravenously administered chyle was rapidly removed from dog plasma. Morris (5) recently reported similar findings in cats. In a previous study in this laboratory, it was found that protamine decreases and heparin increases the rate of removal of intravenously injected chylomicron emulsions from rat plasma (6). It was suggested that the heparin-activated lipolytic enzyme, then termed lipemia clearing factor, which catalyzes the hydrolysis of the triglyceride moiety of chylomicra and low-density lipoproteins, might be involved in fat transport.[2] The purpose of the present study has been to delineate further the mechanisms by which chylomicra are removed from the plasma. To this end, chylomicra, isolated from thoracic duct lymph of dogs fed sodium palmitate-1-C^{14}, have been transfused into recipient dogs. The possible participation of plasma unesterified fatty acids and of various lipoprotein fractions in their transport has been examined. The results obtained strengthen the view that lipoprotein lipase is involved in chylomicron removal and cast doubt on the essentiality of the other plasma lipoproteins in this process in the dog.

METHODS

Mongrel dogs of both sexes fed a stock diet were used. Thoracic duct fistulae were prepared in post-absorptive animals according to the technique of Markowitz (8)

[1] Present Address: Department of Medicine, University of California Medical Center, San Francisco, Calif.

[2] This view has been strengthened by Korn's demonstration of the same enzyme in certain tissues of animals which have not received heparin (7). Because of its specificity, he has termed the enzyme "lipoprotein lipase."

under Sodium Pentothal® anesthesia. All tributaries of the left external jugular vein except the thoracic duct were ligated and the vein ligated and divided high in the neck. The proximal end of the vein was exteriorized and a large polyethylene catheter inserted. When the animal had recovered from the anesthesia he was placed upright in a sling and the clear lymph collected dropwise in a large centrifuge tube. When a lymph flow of 10 to 20 ml. per hour had been established, the animal was given a tracer amount of sodium palmitate-1-C^{14} (100–200 μC.) mixed with 10 to 30 ml. of cream containing 36 gm. fat per 100 ml. by stomach tube. Hydration was maintained with skimmed milk and water. The lymph became turbid within one hour after administration of the cream and cleared again in 10 to 20 hours. After each hour the lymph collected was stored at 3° C. and allowed to clot. The clot was removed by centrifugation and the lymph stored at 3° C. Within 24 hours, the chylomicra were isolated from the lymph as follows. The serum was centrifuged at 80,000 × g. for 30 minutes in the 30 rotor of the Spinco Model L ultracentrifuge at 15° C. The bulk of the chylomicra were packed in a white, butter-like layer at the top of the centrifuge tube with a small amount still dispersed in the upper half of the tube. The packed material was re-emulsified in 0.15 M sodium chloride solution by repeatedly aspirating it through a No. 24 hypodermic needle. The resulting emulsion contained practically no particles over 1 micron diameter on dark field microscopic examination. Recentrifugation of such emulsions can be carried out with little or no change in chemical composition or ease of emulsification. An aliquot of the emulsion was extracted in ethanol-acetone (1:1) for chemical analysis and counting.

Two adult mongrel female dogs weighing 6 kg. were selected to receive the chylomicron emulsions. One dog was used twice; on the first occasion fasted 20 hours (Exper. 1), and two months later, fasted 6 hours (Exper. 2). The other dog was used only once following a 20-hour fast (Exper. 3). The dogs were given sufficient sodium pentobarbital intravenously to induce basal anesthesia (15 to 20 mg. per kg.). During the experiment they received sufficient diethyl ether by inhalation to maintain light anesthesia. Physiologic saline infusions were begun in both femoral veins and maintained at a rate of approximately 0.5 ml. per minute. The freshly prepared chylomicron emulsion (22 to 36 ml.), containing 2.0 to 3.6 gm. total lipid, was injected intravenously. Twenty-ml. blood samples, mixed with 1.5 mg. sodium oxalate per ml. and chilled immediately in ice, were withdrawn at intervals until the plasma was visibly clear, and for some time thereafter. An aliquot of each blood sample was taken for hematocrit determination. The remainder was centrifuged at 3° C. and the plasma removed.

TABLE I

Ultracentrifugally separated lipoprotein fractions

Fraction	Nomenclature	Density	Centrifugal conditions
I	Chylomicra	<1.006	105,000×g.—30 min.
II	Low-density lipoproteins (Sf >10)	<1.019	114,000×g.—15 hr.
III	Beta lipoproteins (Sf 0–10)	1.019–1.063	114,000×g.—15 hr.
IV	Alpha lipoproteins (high density)	1.063–1.21	114,000×g.—22 hr.
V	Residual serum proteins and UFA	>1.21	114,000×g.—22 hr.

Separation of chylomicra from plasma

Aliquots of the plasma were centrifuged immediately at 105,000 × g. for 30 minutes at 3° C. The chylomicra were then packed in a butter-like layer at the top of the tube. The perfectly clear, infranatant plasma was carefully removed by suction through a long needle and centrifuged again under the same conditions. Only a trace of turbid supernatant material was visible after this second centrifugation. The clear, infranatant plasma was removed as before. Prior experiments demonstrated that this chylomicron-free plasma contained essentially all the plasma lipoproteins normally present in post-absorptive dog plasma. The plasma unesterified fatty acids (UFA) were immediately extracted from a two-ml. aliquot of the chylomicron-free plasma (vide infra). The impure chylomicra contained in the butter-like layer from the first centrifugation were re-emulsified in physiologic saline and centrifuged for 15 hours at 114,000 × g. in the 40.3 ultracentrifuge rotor. The chylomicra were concentrated at the top of the tube and the other serum protein constituents concentrated at the bottom. The tube was sliced in a tube slicer and the upper layer emulsified and transferred quantitatively to a 5-ml. volumetric flask (Fraction I).

Lipoprotein fractionation

The remainder of the chylomicron-free plasma was used for the quantitative ultracentrifugal separation of four lipoprotein fractions (9). The densities and nature of the resulting fractions are given in Table I.

Chemical analyses

The plasma and its fractions were extracted in ethanol-acetone (1:1) and analyzed for total cholesterol (10) and lipid phosphorus (11). Phospholipid was estimated by multiplying lipid phosphorus by the factor 25. The plasma and Fraction I were also analyzed for total lipid (12) and free cholesterol (10); and triglyceride was calculated by difference (12). Protein content of the ethanol-acetone insoluble material was determined in the chylomicron samples by the biuret method (13). UFA's were extracted in diethyl ether by the method of Davis (14), in which the plasma is partitioned between the ether and an aqueous phase saturated with so-

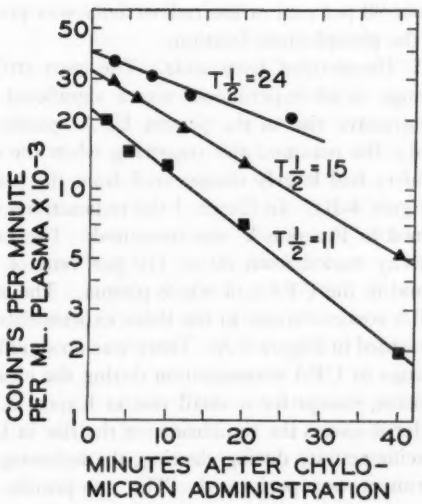


FIG. 1. DISAPPEARANCE OF RADIOACTIVITY FROM DOG PLASMA AFTER INTRAVENOUS ADMINISTRATION OF LABELED CHYLOMICRA

●, ▲, and ■ = Experiments 1, 2, and 3 respectively.

dium sulfate and containing sodium dodecyl sulfate. The ether phase contains all the UFA's, practically all the cholesterol and triglycerides, but almost none of the phospholipids. The ether phase was evaporated to dryness under nitrogen at room temperature, the lipids dissolved in n-heptane, and the UFA's separated from the neutral lipids by the method of Borgström (15). The fatty acids were determined by electrometric titration between pH 6.0 and 10.0.[3] Phospholipids were separated from other lipids of the chylomicra on a silicic acid column according to Borgström (16).

Radioactivity determinations

Samples were prepared in duplicate for assay of radioactivity by mixing suitable aliquots of the lipid extracts with sufficient olive oil to give a total weight approximating 10 mg. These aliquots were evaporated on lens paper in aluminum planchets. Radioactivity was assayed with a Robinson flow gas counter and the results corrected to an absorption weight of 6.2 mg. per cm^2.

All experimental data for the various fractions and the plasma, with the exception of specific activities, have been corrected to milligrams or counts per minute per ml. of native plasma.

RESULTS

1. *Chylomicron composition.* The chemical composition of the three isolated chylomicron preparations is summarized in Table II. The average molecular weight of the fatty acids in preparation No. 1 was estimated to be 260. This figure was determined by dividing the mg. of fatty acid as determined chemically by the concentration in milliequivalents as determined titrimetrically after saponification. The phospholipid fraction contained only 1.5 per cent of the total radioactivity in preparation No. 1. It has been assumed, therefore, in subsequent calculations that all the radioactivity in the chylomicra was contained in triglyceride fatty acids.

2. *In vitro transfer of chylomicron radioactivity.* In order to determine the extent to which transfer of chylomicron radioactivity occurred in the absence of the clearing process, dog chylomicra were incubated *in vitro* with normal dog plasma for 90 minutes at 37° C. Lipoprotein fractions were then separated and total radioactivity in each fraction determined. There was 2.0 per cent of the activity in Fraction II, 0.4 per cent in Fraction III, 0.4 per cent in Fraction IV, and 0.2 per cent in Fraction V.

3. *Removal of chylomicra from the plasma.* The rates of disappearance of radioactivity from the whole plasma are shown in Figure 1. Chylomicron (Fraction I) radioactivity disappeared at the same rate. The removal appeared to follow a first order reaction. The deviations from linearity on the graph are probably due in part to variations in plasma volume secondary to the repeated bleedings, since the hematocrit fell from an average of 45 to 37 during the clearing process. Similar straight lines were obtained when the disappearance of triglycerides and phospholipids from the chylomicra isolated from each sample were plotted on a semilogarithmic scale. The triglyceride and phospholipid half-lives in the three experiments

TABLE II

Chemical composition of dog lymph chylomicra

Substance	Per cent by weight (range)
Cholesterol esters	1.5–2.0
Free cholesterol	0.7–1.0
Phospholipids	8.2–8.9
Triglycerides	86.3–86.5
*Unesterified fatty acids	0.2
Protein	2.2–2.5

[3] We are indebted to Miss Amelia Cherkes for performing these titrations.

* Based on per cent radioactivity in isolated unesterified fatty acids in one experiment.

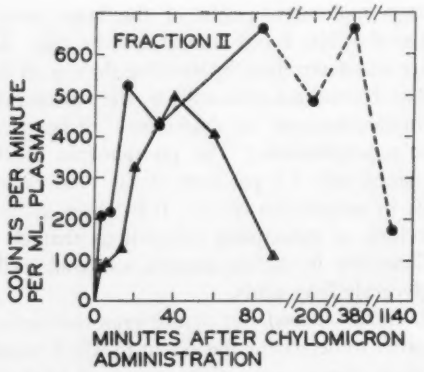


FIG. 2. RADIOACTIVITY IN FRACTION II LIPOPROTEINS FOLLOWING INTRAVENOUS ADMINISTRATION OF LABELED CHYLOMICRA

● and ▲ = Experiments 1 and 2, respectively.

were 28 and 28, 18 and 17, and 11 and 12 minutes, respectively. There was insufficient cholesterol and protein in these preparations to permit accurate determination of half periods. The volume of distribution of the radioactivity at time zero was 50 to 73 ml. per kg., corresponding to accepted values for plasma volume in the dog (17).

4. *Analysis of other lipoprotein fractions.* The appearance of labeled fat in the other fractions during clearing was measured after two separate chylomicron infusions (Figure 2). Radioactivity in Fraction II rose during the early phase of clearing in both experiments. However, the maximum total radioactivity in this fraction never exceeded 2 per cent of the total plasma radioactivity at time zero and there was no detectable change in the total lipid or lipid phosphorus concentrations in this fraction during the clearing process. The pertinent data regarding the S_f 0–10 (Fraction III) and alpha (Fraction IV) lipoproteins are shown for Exper. 1 in Figure 3. The data for Exper. 2 were comparable. It is apparent that only small amounts of radioactivity appeared, and cholesterol and phospholipid concentrations varied little in these fractions during clearing. On the other hand, radioactivity and phospholipid concentrations did rise strikingly in both of these fractions several hours after clearing was complete. When aliquots of the alpha lipoproteins from four serial blood samples (Exper. 2) were extracted by the Davis procedure, it was found that about 90 per cent of the radioactivity was present in the phospholipid fraction.

5. *Unesterified fatty acids.* The most striking change in all experiments was a significant and progressive rise in the plasma UFA specific activity, the maximal rise occurring when the chylomicra had largely disappeared from the plasma (Figure 4-B). In Exper. 1 the radioactivity contained in Fraction V was measured. The radioactivity varied from 80 to 110 per cent of that found in the UFA's of whole plasma. The serial UFA concentrations in the three experiments are presented in Figure 4-A. There was no significant change in UFA concentration during the clearing process, except for a small rise in Exper. 3. In order to assess the significance of the rise in UFA specific activity during clearing, the following experiment was performed. The non-protein solvent density of a 15-ml. sample of fresh dog serum was brought to 1.21 by addition of potassium bromide. The lipoproteins were removed after centrifuging at 114,000 × g. for 22 hours, and the infranatant solution thoroughly dialyzed against physiologic saline. Three mg. of sodium palmitate-1-C^{14} (2.3×10^6 c.p.m.) were dissolved in 10 ml. of physiologic saline with warming to give

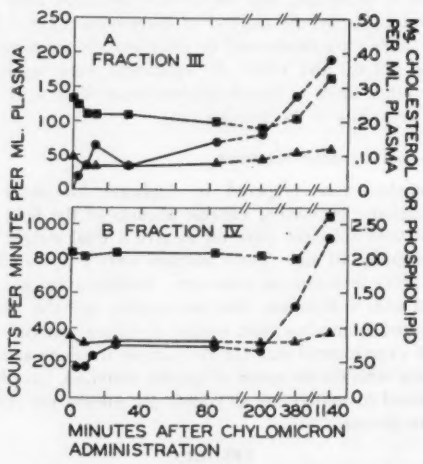


FIG. 3. RADIOACTIVITY AND LIPID CONCENTRATIONS IN FRACTION III AND IV LIPOPROTEINS FOLLOWING INTRAVENOUS ADMINISTRATION OF LABELED CHYLOMICRA IN EXPERIMENT 1

● = radioactivity, ▲ = total cholesterol, and ■ = phospholipids.

an opalescent solution. This solution was mixed with the dialyzed, lipoprotein-free protein solution. A clear solution resulted, with no evidence of turbidity or precipitate, even in the cold. It was previously established by ultracentrifugal fractionation that sodium palmitate added in this way is completely bound to the serum proteins. After removal of aliquots for assay of radioactivity, the remainder of the solution was injected intravenously in a 10-kg. dog fasted for 20 hours. Anesthesia was given as described previously. Five-ml. serial blood samples were withdrawn at close intervals for 11 minutes, and the plasma removed. Plasma aliquots were extracted by the Davis method (14) and the UFA's separated in selected samples (15). The half-life of UFA radioactivity was found to be extremely short, about two minutes (Figure 5). In the samples taken at 1 and 11 minutes, practically all the radioactivity present was in the form of unesterified fatty acids.

6. *Radioactivity in red blood cells.* In Experiment 2, aliquots of the cells remaining after removal of the plasma were washed three times with physiologic saline, lyzed in distilled water, and plated directly on aluminum planchets for assay of their radioactivity. No significant radioactivity was found.

7. *Intravascular lipolysis during clearing.* In Experiments 2 and 3, several aliquots of lipemic plasma, taken following the chylomicron infusion, were incubated at 37° C. for two hours and serial measurements of optical density at 500 mμ made in a Coleman Jr. spectrophotometer. In no instance was there a fall in optical density greater than 0.02 unit during the period of incubation. These measurements were completed within four hours after taking the blood sample, and the plasmas were kept at 3° C. until the time of incubation. These *in vitro* studies suggest a lack of intravascular lipolysis *per se* during the clearing process.

DISCUSSION

Dog chylomicra are composed chiefly of triglycerides. Their composition is similar to that of human chylomicrons.[4] In our experiments, approximately 97 per cent of the chylomicron radioactivity following feeding of sodium palmitate-1-C^{14} was contained in triglycerides. In calculating the triglyceride fatty acid specific activity, the assumption that all the radioactivity was contained in the triglycerides therefore introduces no significant error. Our finding of 1.5 per cent of the radioactivity in the phospholipid moiety of the chylomicra is in good agreement with that of Bloom, Chaikoff, Reinhardt, and Dauben (1), who fed sodium palmitate-1-C^{14} to rats and found not more than 4 per cent of the label in the phospholipid fatty acids of thoracic duct lymph. While not measured, cholesterol esters could not have accounted theoretically for more than 1 per cent of the radioactivity found in dog chylomicra. The paucity of UFA's in the chylomicra confirms Borgström's finding in rats (15). The small quantity we found could have resulted from *in vitro* lipolysis.

The rapid turnover of chylomicra in dog plasma

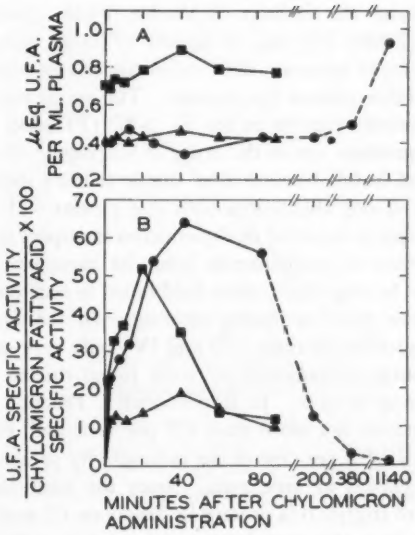


FIG. 4. A. PLASMA UNESTERIFIED FATTY ACID CONCENTRATIONS AFTER INTRAVENOUS ADMINISTRATION OF LABELED CHYLOMICRA

B. SPECIFIC ACTIVITY OF UNESTERIFIED FATTY ACIDS AFTER INTRAVENOUS ADMINISTRATION OF LABELED CHYLOMICRA EXPRESSED AS PER CENT OF THE SPECIFIC ACTIVITY OF THE FATTY ACIDS IN THE ADMINISTERED CHYLOMICRON TRIGLYCERIDES

●, ▲, and ■ = Experiments 1, 2, and 3 respectively.

[4] Bragdon, J. H., Havel, R. J., and Boyle, E., data to be published. It should be noted that in the present experiments no attempt was made to remove traces of plasma proteins from the preparations. Therefore, the protein content is probably lower than that reported.

confirms the findings of Marble, Field, Drinker, and Smith (4) and of Morris (5). In spite of this rapid turnover, little radioactivity appeared in the other plasma lipoproteins. The maximum radioactivity present in the $S_f > 10$ (Fraction II) lipoproteins was of the order of magnitude of that found in this fraction after simple *in vitro* incubation of dog chylomicra with dog plasma. If this fraction is involved in chylomicron transport as an acceptor of chylomicron lipid, its turnover rate must be very rapid, since lipid failed to accumulate in this fraction during clearing. In the major lipoprotein fractions (III and IV) only very small amounts of radioactivity were found during the clearing process. In Experiment 1, Fraction III contained not more than 0.2 per cent and Fraction IV 0.8 per cent of the radioactivity present in the plasma at time zero. Since the total quantity of triglyceride present in Fractions III and IV was low, the measurement of specific activities was impossible. However, the radioactivity found in these fractions during clearing was similar to that found after *in vitro* incubation of chylomicra with normal dog plasma. The radioactivity found, both *in vitro* and *in vivo*, may have been due to exchange of phospholipids between the chylomicra and the other lipoproteins, since, in Fraction IV, the bulk of the radioactivity was shown to be associated with the phospholipids. The late rise in radioactivity and phospholipid concentrations in Fractions III and IV must represent new lipoprotein formation, perhaps in response to the appreciable blood loss that occurred. The data presented do not support the concept that other lipoproteins are involved in the removal of chylomicra from the plasma. It should be pointed out that the present experiments do not bear upon their possible participation in the *formation* of the chylomicron (18).

Of great interest was the rise in specific activity of the unesterified fatty acids during clearing. This finding suggests that there is an intimate relationship between removal of chylomicra and the hydrolysis of their constituent triglycerides. It is possible that this rise resulted from intravascular lipolysis, but we were unable to observe evidence of continued lipolysis *in vitro* in these experiments. Robinson, Jeffries, and French have reported that *in vitro* lipolysis occurs in the plasma of one strain of rats after fat feeding, and have calculated that the extent of this lipolysis is sufficient to account for the rate of disappearance of lipemia in these animals (19). We have been unable to reproduce this phenomenon in two other strains of rats.

It is evident from the high specific activity of the plasma UFA's during chylomicron removal that at least part of the UFA's newly released by hydrolysis is returned rapidly to the blood stream. They are apparently transported in the usual manner, namely, complexed with serum albumin, for essentially all the plasma UFA radioactivity was found in the albumin-containing 1.21 density infranates. Although the half-life of the plasma UFA's, and thus the proportion of the cleared triglyceride which was retransported as fatty acid could not be determined during the experiments involving chylomicron clearance, the constancy of the UFA concentration and the high UFA specific activity indicate that UFA transport during the clearing interval is probably extremely active. The magnitude of the UFA turnover possible during clearing may be appreciated by considering the results in Experiment 1. Approximately 2,500 mg. of triglyceride fatty acid disappeared from the plasma in 80 minutes, an average of 30 mg. per minute. If the plasma volume is assumed to be 300 ml., the quantity of plasma UFA's present at any time was about 30 mg., since their concentration remained constant at about 0.1 mg. per ml. plasma during clearing. Thus the plasma UFA's would have to be turned over at least every minute, were all the cleared triglyceride re-transported as fatty acid in this interval. When actually determined in a separate, anesthetized, fasting dog, the plasma UFA half-life was found to be two minutes. This suggests the presence of a mechanism capable of accomplishing the rapid re-transport of much of the chylomicron triglyceride hydrolyzed during clearing. The extreme lability of plasma UFA's in response to various metabolic alterations demonstrated by Gordon and Cherkes (20) also indicates that their turnover can be very rapid under several conditions.

While our findings indicate that the lipolysis associated with the removal of chylomicra from the plasma did not occur in the circulating blood, the actual site of chylomicron removal remains to be

determined. The reticulo-endothelial cells present in the vascular wall of certain organs, or the capillary cells themselves, are suggested as possible sites of removal. Friedman, Byers, and Rosenman (21) have presented evidence that newly absorbed cholesterol is removed by the Kupffer cells of the liver in the rat. The problem of the sites of chylomicron removal is the subject of current investigation in our laboratory.

The close association observed between chylomicron clearance and lipolysis supports the hypothesis that the heparin-activated enzyme, lipoprotein lipase, is involved in the removal of exogenous lipid from the plasma. The observation that protamine sulfate inhibits the *in vitro* activity of lipoprotein lipase (22) can thus explain its retarding effect on chylomicron removal. This retardation of *in vivo* clearing by protamine indicates that lipolysis must be a rate-limiting step in this process.

SUMMARY

Chylomicra have been isolated from the thoracic duct lymph of dogs fed sodium palmitate-1-C^{14}. Their chemical composition was similar to that reported for humans, with approximately 86 per cent triglyceride by weight. After intravenous injection in recipient dogs, the chylomicra disappeared exponentially, with a half-life of 15 to 24 minutes. The triglyceride and phospholipid moieties of the chylomicra disappeared from the plasma at the same rate. Small quantities of radioactivity were found in three ultracentrifugally separated lipoprotein fractions during clearing, but the physiologic significance of this finding in relation to the removal of chylomicra from dog plasma is questioned. In fasted dogs, the specific activity of the plasma unesterified fatty acids rose progressively during clearing and reached a level exceeding 50 per cent of the specific activity of the transfused triglyceride fatty acids. This close association between the removal of chylomicra and the release of unesterified fatty acids into the plasma supports previous work suggesting that the lipolytic enzyme, lipoprotein lipase, is involved in the removal of chylomicra from the plasma. Plasma unesterified fatty acids had a half-life of about two minutes in dogs under the conditions of these experiments. This is interpreted to indicate that an appreciable portion of the cleared chylomicron triglyceride may be re-transported through the plasma as unesterified fatty acid during the clearing process.

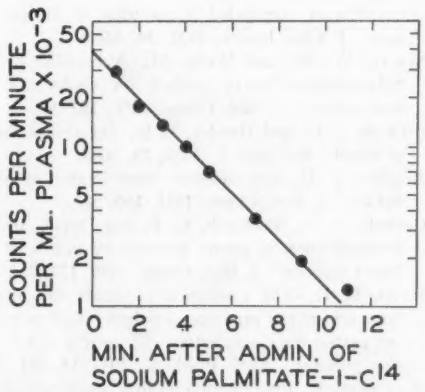


FIG. 5. DISAPPEARANCE OF RADIOACTIVITY FROM DOG PLASMA FOLLOWING INTRAVENOUS ADMINISTRATION OF SODIUM PALMITATE-1-C^{14} (23.5×10^8 COUNTS PER MINUTE) COMPLEXED WITH LIPOPROTEIN-FREE DOG SERUM PROTEINS

REFERENCES

1. Bloom, B., Chaikoff, I. L., Reinhardt, W. O., and Dauben, W. G., Participation of phospholipides in lymphatic transport of absorbed fatty acids. J. Biol. Chem., 1951, **189**, 261.
2. Bergström, S., Borgström, B., and Rottenberg, M., Intestinal absorption and distribution of fatty acids and glycerides in the rat; metabolism of lipids 3. Acta physiol. Scandinav., 1952, **25**, 120.
3. Jones, H. B., Gofman, J. W., Lindgren, F. T., Lyon, T. P., Graham, D. M., Strisower, B., and Nichols, A. V., Lipoproteins in atherosclerosis. Am. J. Med., 1951, **11**, 358.
4. Marble, A., Field, M. E., Drinker, C. K., and Smith, R. M., The permeability of the blood capillaries to lipoids. Am. J. Physiol., 1934, **109**, 467.
5. Morris, B., The interrelationships of the plasma and lymph lipide fractions before and during fat absorption. Australian J. Exper. Biol. & M. Sc., 1954, **32**, 763.
6. Bragdon, J. H., and Havel, R. J., *In vivo* effect of anti-heparin agents on serum lipids and lipoproteins. Am. J. Physiol., 1954, **177**, 128.
7. Korn, E. D., Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. J. Biol. Chem., 1955, **215**, 1.
8. Markowitz, J., Experimental Surgery, Including Surgical Physiology. 3rd ed., Baltimore, Williams & Wilkins Co., 1954,, p. 648.
9. Havel, R. J., Eder, H. A., and Bragdon, J. H., The distribution and chemical composition of ultra-

centrifugally separated lipoproteins in human serum. J. Clin. Invest., 1955, **34**, 1345.
10. Sperry, W. M., and Webb, M., A revision of the Schoenheimer-Sperry method for cholesterol determination. J. Biol. Chem., 1950, **187**, 97.
11. Stewart, C. P., and Hendry, E. B., The phospholipins of blood. Biochem. J., 1935, **29**, 1683.
12. Bragdon, J. H., Colorimetric determination of blood lipides. J. Biol. Chem., 1951, **190**, 513.
13. Gornall, A. G., Bardawill, C. J., and David, M. M., Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 1949, **177**, 751.
14. Davis, B. D., The estimation of small amounts of fatty acid in the presence of polyoxyethylene sorbitan partial fatty acid esters ("Tween") and of serum proteins. Arch. Biochem., 1947, **15**, 351.
15. Borgström, B., Investigation on lipid separation methods. Separation of cholesterol esters, glycerides and free fatty acids. Acta physiol. Scandinav., 1952, **25**, 111.
16. Borgström, B., Investigation on lipid separation methods. Separation of phospholipids from neutral fat and fatty acids. Acta physiol. Scandinav., 1952, **25**, 101.
17. Gregersen, M. I., Boyden, A. A., and Allison, J. B., Direct comparison in dogs of plasma volume measured with T-1824 and with antigens. Am. J. Physiol., 1950, **163**, 517.
18. Korn, E. D., Clearing factor, a heparin-activated lipoprotein lipase. II. Substrate specificity and activation of cocoanut oil. J. Biol. Chem., 1955, **215**, 15.
19. Robinson, D. S., Jeffries, G. H., and French, J. E., Studies on the interaction of chyle and plasma in the rat. Quart. J. Exper. Physiol., 1954, **39**, 165.
20. Gordon, R. S., and Cherkes, A., Unesterified fatty acid in human blood plasma. J. Clin. Invest., 1956, **35**, 206.
21. Friedman, M., Byers, S. O., and Rosenman, R. H., Observations concerning the production and excretion of cholesterol in mammals. XII. Demonstration of the essential role of the hepatic reticuloendothelial cell (Kupffer cell) in the normal disposition of exogenously derived cholesterol. Am. J. Physiol., 1954, **177**, 77.
22. Brown, R. K., Boyle, E., and Anfinsen, C. B., The enzymatic transformation of lipoproteins. J. Biol. Chem., 1953, **204**, 423.

TRACER STUDIES OF THE ABSORPTION AND FATE OF STEROID HORMONES IN MAN [1]

By LEON HELLMAN, H. LEON BRADLOW, E. L. FRAZELL, AND T. F. GALLAGHER

(From the Divisions of Biophysics, Steroid Metabolism and Biochemistry, Sloan-Kettering Institute for Cancer Research, the Head and Neck Service, Memorial Center and the Sloan-Kettering Division, Cornell University Medical College, New York, N. Y.)

(Submitted for publication December 9, 1955; accepted May 17, 1956)

The steroid hormones testosterone, hydrocortisone and corticosterone present certain paradoxical physiological problems in their transport and metabolism in the organism. Thus hydrocortisone is highly effective orally whereas testosterone has little biological activity when fed. Insofar as man and the rodent are concerned, there appears to be no appreciable species difference in the response to these two hormones when given by mouth. On the other hand, there is a striking difference in the manner in which these two species eliminate the end product of hormone metabolism (1–3) and, in addition, dissimilar compounds are formed and excreted by each. A further species difference exists in that hydrocortisone is the principal product of the adrenal secretion in man, whereas in the rat corticosterone appears to be the major component (4).

Since an interpretation of these differences might give a deeper insight into the mechanism of hormonal action, an exploration of the problems implicit in these facts was undertaken. First the possibility was entertained that corticosterone might behave like a hormone "foreign" to the human and would, as a consequence, differ from the "normal" secretion, hydrocortisone, in either the rate or route of elimination of its metabolites. Second, cortisone acetate was compared with hydrocortisone and corticosterone to establish, by the same criteria, whether similar processes are operative with the biologically active ester as with the free hormones. Third, testosterone was given by both the oral and intravenous route in order to have a comparison of a chemically similar steroid hormone, differing in biological activity, with the two adrenal hormones. Fourth, the absorption of hydrocortisone and testosterone from the intestinal tract was studied in human subjects with the thoracic duct cannulated. Fifth, the importance of biliary excretion in the human was evaluated in patients with complete biliary fistulas. The results reported are of interest for the comparative physiology of the hormones since the findings obtained in other species can now be related to the sequence of events observed with the human.

EXPERIMENTAL

The subjects studied were patients in a metabolic ward. The pertinent clinical data concerning these patients are summarized in Table I. The three subjects with bile fistula, FBT-1, T-3, B-1, were studied in the interval between the two stages of an operative attempt to correct bile duct obstruction as the result of a tumor. In each case the fistula was deliberately established during the first operative procedure by complete diversion of bile flow to the exterior through an indwelling cannula. The completeness of the fistula was confirmed by the absence of bile pigments in the feces. The subjects with thoracic duct fistulas were operated upon for the purposes of this study and a polyethylene cannula was inserted through a supraclavicular incision into the left thoracic duct (5). The patients were fed a meal containing fat before the operation in order to distend the thoracic duct to facilitate insertion of the cannula.

During the early portion of each study, urine specimens were obtained through a catheter. Complete collections of urine and feces were continued for varying periods after the administration of the labelled material. Blood samples were collected with heparin as the anticoagulant. Radioactivity assays were carried out by the two procedures previously described (6) both in the infinitely thick and in the infinitely thin regions. The values reported are the average of the two counting techniques, which agreed within less than 5 per cent. Conjugated and free urinary steroids were separated by the procedure of de Courcy, Bush, Gray, and Lunnon (7);

[1] This investigation was carried out under contract AT30-1 910 with the United States Atomic Energy Commission and supported in part by a research grant (C-440) from the National Cancer Institute of the National Institutes of Health of the United States Public Health Service, and a research grant from the American Cancer Society.

TABLE I

Clinical data

Subject	Experiment	Sex	Age	Diagnosis	Remarks
FT-1	1, 2, 3, 6D, 7B	F	65	Diabetes. Kimmelstiel-Wilson syndrome	Minimal edema. Proteinuria. Good renal function.
F-5	4	M	39	Multiple sclerosis	Good general condition. Normal liver and kidney function.
Eac-1	5	F	70	Carcinoma of breast	Good general condition. Normal liver and kidney function.
T-1	6A	M	44	Aplastic anemia	Good general condition. Required frequent transfusions.
T-2	6B, 6C	M	70	Carcinoma of prostate	Good general condition. Normal liver and kidney function.
T-4	7A	M	31	Normal	Excellent health.
F-6	8	M	69	Carcinoma of tongue	Poor general condition. Thoracic duct cannulated.
FT-2	9, 10	M	40	Carcinoma of tongue	Poor general condition. Thoracic duct cannulated.
FBT-1	11, 12, 13A, 14	F	57	Carcinoma of gall bladder	Fair general condition. Complete bile fistula.
T-3	13B	M	60	Carcinoma of stomach	Fair general condition. Complete bile fistula.
B-1	15	F	60	Carcinoma of gall bladder	Fair general condition. Complete bile fistula.

radioactivity in the blood was measured as previously described (6).

The testosterone-4-C^{14} [2] had a specific activity of 0.52 μc. per mg. Reverse isotopic dilution established that the material was at least 91 per cent pure and that Δ^4-androstene-3,17-dione-4-C^{14} was a minor impurity. The hydrocortisone-4-C^{14}, cortisone-4-C^{14} acetate and corticosterone-4-C^{14} were obtained from the Endocrinology Study Section of the United States Public Health Service (Dr. Sam Hall) and had specific activities of 4.4 μc. per mg., 1.2 μc. per mg., and 4.4 μc. per mg., respectively.[3] For intravenous administration, a sterile solution was freshly prepared as described previously (6) and a known quantity was then infused over a 30-minute period. In those experiments where the hormones were administered orally, a solution, prepared in an identical manner with that used for the intravenous doses, was ingested by the patients who had been fasted for at least 12 hours. The patients with the thoracic duct fistulas received the steroids orally by stomach tube in a milk and cream suspension.

RESULTS

Oral hydrocortisone

Three studies, experiments 2, 3, and 4, are reported in Table II. The cumulative excretion of radioactivity in urine and the rate of excretion of radioactivity derived from experiment 4 (Subject F-5) are shown in Figures 1 and 2. For convenience of interpretation, typical curves after intravenous administration of hydrocortisone taken from the previous report (6) are also shown in these figures. Subjects FT-1 (two experiments) and F-5 excreted from 61 to 70 per cent of the radioactivity of the hydrocortisone within 24 hours. The results after intravenous administration of hydrocortisone to the same subject FT-1 in experiment 1 are quite similar to those of the oral experiments.

Cortisone acetate

In experiment 5, cortisone acetate was given orally to subject Eac-1. Figures 1 and 2 indicate that the initial rate of excretion was somewhat different than that of free hydrocortisone. However, the cumulative excretion of radioactivity from the esterified steroid was not significantly different from that observed after the administration of free hydrocortisone; 61 per cent was excreted in the first day with a total of 75 per cent appearing in the urine at the end of 3 days.

[2] Synthesized by Dr. R. B. Turner of the Rice Institute, Houston, Texas, and generously furnished by him.

[3] The hydrocortisone-4-C^{14} was found to be 93 to 94 per cent pure by several investigators using a variety of techniques. The corticosterone-4-C^{14} was about 96 to 97 per cent pure. Cortisone-4-C^{14} acetate was about 94 per cent pure and contained about 4 per cent of the free alcohol. The information about the purity of these compounds was a personal communication from the Endocrinology Study Section.

TABLE II

Excretion of radioactivity following administration of C^{14} labelled steroids to intact subjects

Experiment	1	2	3	4	5	6D*	7A	7B
Subject	FT-1	FT-1	FT-1	F-5	Eac-1	FT-1	T-4	FT-1
Substance	Hydro-cortisone	Hydro-cortisone	Hydro-cortisone	Hydro-cortisone	Cortisone Acetate	Testos-terone	Testos-terone	Testos-terone
Dose (*mg.*)	0.15	0.11	0.18	0.20	0.25	2.5	1.5	2.7
Dose (*μc.*)	0.67	0.54	0.86	0.94	0.96	1.6	0.77	1.7
Route	I.V.	Oral	Oral	Oral	Oral	I.V.	Oral	Oral
Time: *hours*	Urinary Radioactivity: Per Cent Dose							
0–1	2.0	3.9	3.3	6.8	2.8	4.5	24.6	12.1
1–2	6.2	5.1	6.5	15.4	4.3	8.3	19.0	11.4
2–3	8.4	6.2	5.9	11.2	4.0	7.3	5.8	5.9
3–4	6.0	5.8	5.2	8.4	5.4	4.0	6.5	4.9
4–5	5.6	4.3	4.1	6.8	4.2	3.6	3.6	3.9
5–6	5.4	4.3	4.6	4.3	5.3	2.2	2.4	3.0
6–12	15.5	17.5	9.0	9.3	17.8	11.3	5.6	11.1
12–18	7.7	12.0	18.4	8.2	10.1	5.7	1.7	4.9
18–24	11.3	7.4	4.6		7.1	2.0	1.5	4.2
24–48	7.5	0	13.8	8.6	11.9	3.6	4.9	5.9
48–72	13.6	0		2.9	1.9		4.6	0
72–96	11.4			3.4	0		3.9	
Total:								
24 hours	68.1	66.5	61.6	70.4	61.0	50.0	75.6	61.4
$T_{\frac{1}{2}}^{2}$: hours†	1.5	2	4	2	3	3	1.5	2
$T_{\frac{1}{2}}^{1}$: hours†	17	10	34	13	13.5	13	35	11

* Experiment 6A, Subject T-1, I.V. Testosterone, 53% excreted in 24 hours.
Experiment 6B, Subject T-2, I.V. Testosterone, 60% excreted in 24 hours.
Experiment 6C, Subject T-2, I.V. Testosterone, 55% excreted in 24 hours.
No fractional urine collections were made in Experiments 6A, B, and C.

† $T_{\frac{1}{2}}^{2}$ describes the rapid component in terms of "half-life" and $T_{\frac{1}{2}}^{1}$, the slow component, of the rate of excretion of hormone metabolites.

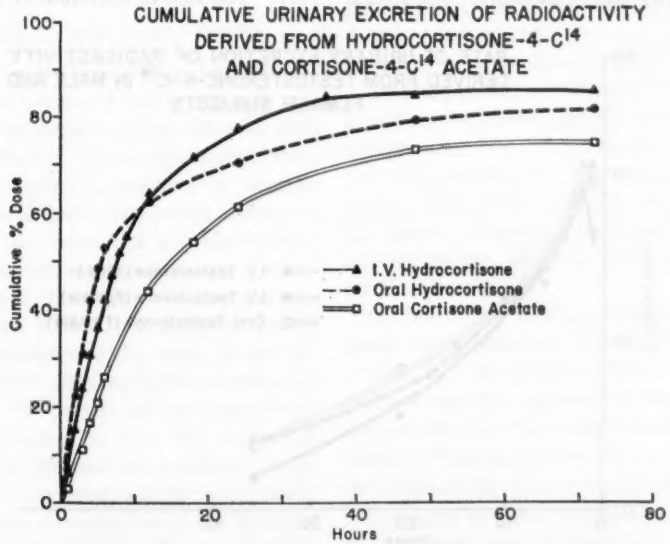


FIG. 1. CUMULATIVE URINARY EXCRETION OF RADIOACTIVITY DERIVED FROM HYDROCORTISONE-4-C^{14} AND CORTISONE-4-C^{14} ACETATE

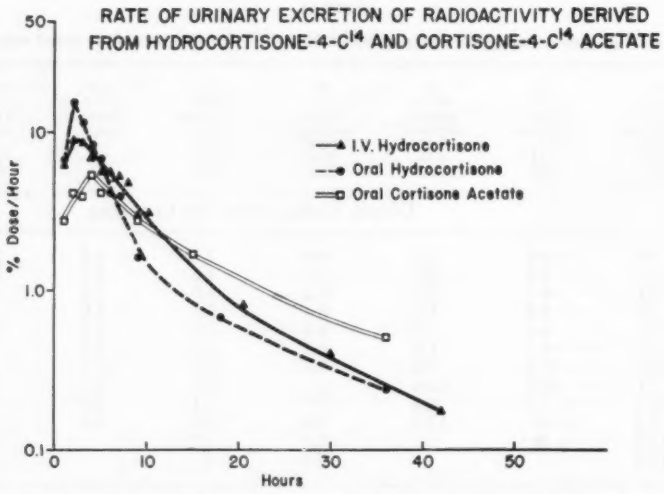


FIG. 2. RATE OF URINARY EXCRETION OF RADIOACTIVITY DERIVED FROM HYDROCORTISONE-4-C^{14} AND CORTISONE-4-C^{14}-ACETATE

Testosterone

Results following the oral and intravenous administration of testosterone-4-C^{14} to male and female subjects are given in Table II and Figure 3. Two male subjects, T-1, T-2, received testosterone intravenously (experiments 6A, 6B, and 6C) and 53 to 60 per cent of the administered radioactivity appeared in the urine within 24 hours. The female subject, FT-1, excreted 50 per cent during the first day following intravenous testosterone (experiment 6D) and 61 per cent of the radioactivity of orally administered testosterone in the first 24-hour period (experiment 7B). A normal male subject, T-4, excreted 76 per cent of oral testosterone radioactivity during the first day (experiment 7A). The rate of excretion of the metabolites of

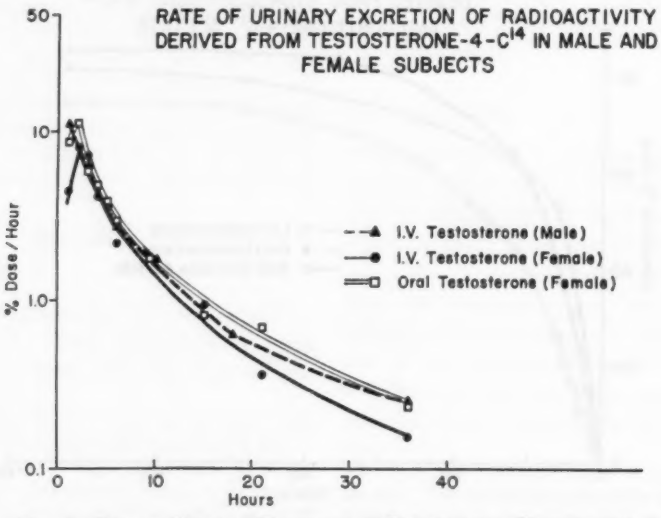

RATE OF URINARY EXCRETION OF RADIOACTIVITY DERIVED FROM TESTOSTERONE-4-C14 IN MALE AND FEMALE SUBJECTS
% Dose/Hour
Hours
I.V. Testosterone (Male)
I.V. Testosterone (Female)
Oral Testosterone (Female)

FIG. 3. RATE OF URINARY EXCRETION OF RADIOACTIVITY DERIVED FROM TESTOSTERONE-4-C^{14} IN MALE AND FEMALE SUBJECTS

testosterone was similar in male and female subjects following oral or intravenous administration, and resembled the rate of excretion after hydrocortisone in both the current studies and those previously reported.

Studies in patients with thoracic duct fistulas

Table III contains the data for experiments 8, 9, and 10 concerning the excretion of metabolites of hydrocortisone and testosterone following the administration of these hormones to subjects with thoracic duct fistulas. In experiment 8, 40 per cent of hydrocortisone radioactivity appeared in the urine within 10 hours and the complete collection of lymph made during this period failed to contain any significant amount of radioactivity. The result was confirmed in another subject, FT-2, in whom 17 per cent of the radioactivity appeared in the urine within 4 hours; during this period no radioactivity was present in lymph. In order to test the possibility that a different route of absorption might be responsible for the biological inactivity of oral testosterone, the same subject, FT-2, received labelled testosterone in experiment 10. Sixty per cent of the metabolites of testosterone appeared in the urine within 24 hours and the complete lymph collection made during that period was devoid of any radioactivity.

TABLE III

Excretion of radioactivity following oral C^{14} labelled steroids in patients with thoracic duct fistulas

Experiment	8	9	10
Subject	F-6	FT-2	FT-2
Substance	Hydrocortisone	Hydrocortisone	Testosterone
Dose (*mg.*)	0.25	0.25	4.2*
Dose (*μc.*)	0.65	0.6	3.0*
Route	Oral	Oral	Oral
Time: *hours*	Urinary Radioactivity: Per Cent Dose		
0–3	13.0	11.2	5.0*
3–4	6.2	6.2	5.0
4–6	5.6		10.0
6–10	15.9		20.0
10–27			20.0
Total	10 hours: 40.7	4 hours: 17.4	27 hours: 60.0
Time: *hours*	Lymph Radioactivity: Per Cent Dose		
0–2	0	0	0
2–4	0	0	0
4–6	0	0	0
6–8	0.3		0
8–10	0		0
10–27			0

* Because of some slight uncertainty in estimating the amount of cream containing the dose of labelled steroid actually received by the patient, the values in this column have been rounded off.

Experiments in patients with biliary fistulas

Table IV and Figures 4 and 5 give the data concerning the administration of hydrocortisone, testosterone and corticosterone to two subjects with complete biliary fistulas. These experiments were done in order to study the presence or absence in man of an enterohepatic circulation, known to occur in rodents, for hormones and metabolites. Following the intravenous administration of small and large doses of hydrocortisone in experiments 11 and 12 to subject FBT-1, about 80 per cent of the radioactivity appeared in the urine in each instance within 24 hours with negligible excretion in the bile. During this period bile was completely diverted to the exterior, so that the absence of radioactivity in the bile and the presence of the expected amount in the urine indicate conclusively that biliary excretion of metabolites of the hormone was negligible in this subject. Experiments 11 and 12 were at different dose levels, 0.25 mg. and 100 mg., respectively, to test whether the capacity of the liver to handle the hormone might be exceeded. The identical results in both experiments indicate that, within these limits, the size of the dose is not critical for the appearance or absence of hormone metabolites in human bile.

Two experiments, 13A and 13B, were done in a male and a female patient with complete biliary fistulas after the administration of testosterone. In both instances the expected amount of radioactivity derived from testosterone was excreted in the urine. Although there was detectable radioactivity in the bile the total excretion by this route was small.

Corticosterone was also administered to two patients with complete bile fistulas (experiments 14 and 15). As shown in Table IV, the amount of radioactivity that appeared in the urine was quite comparable with that observed after the administration of hydrocortisone; only a small fraction of the radioactivity was recovered in the bile.

The data on radioactivity found in blood after the different steroid hormones are summarized in Table V. The "free" and "conjugated" radio-

TABLE IV

Excretion of radioactivity following C^{14} labelled steroids in patients with biliary fistulas

Experiment	11	12	13A*	14	15
Subject	FBT-1	FBT-1	FBT-1	FBT-1	B-1
Substance	Hydrocortisone	Hydrocortisone	Testosterone	Corticosterone	Corticosterone
Dose (*mg.*)	0.25	100	4.2	0.6	0.6
Dose (*μc.*)	0.85	0.68	3.0	0.96	1.0
Route	I.V.	I.V.	I.V.	I.V.	I.V.
Time: *hours*			Urine: Per Cent Dose		
0–1	13.1	15.3	9.5	3.3	6.0
1–2	13.8	12.5	15.1	10.7	11.2
2–3	9.9	10.0	10.0	15.8	9.1
3–4	15.5			12.4	8.4
4–5		22.6	15.1	8.8	13.6
5–6	21.2			6.8	8.0
6–12		15.3	7.5	13.2	15.2
12–18	6.7	4.1	6.9	10.3	3.3
18–24	1.9	1.8	2.1	3.9	2.3
24–48	3.5	4.1	6.5	2.6	2.3
48–72	0	1.3	4.2	0.8	0.7
72–96	0		1.6		
Total: 24 hours	82.1	81.6	67.	85.2	77.1
$T_{\frac{1}{2}}^{2}$: hours†	3.5	2.5	2.5	1.5	1.5
$T_{\frac{1}{2}}^{1}$: hours†	18	14	17	6	9
Time: *hours*			Bile: Per Cent Dose		
0–2	0	0	0.05	0	0
2–4	0	0.4	0.2	0	0.2
4–6	0	0.3	0.1	0.3	0.5
6–12	0.3	0	0.1	0.1	1.0
12–18	0.2	0	0.3	0.1	0.9
18–24	0	0	0.4	0.3	0.4
24–48	0	0	0.4	0.2	0.1
48–72	0	0	0.7	0.1	0
72–96	0	0	0.5		0
Time: *days*			Feces: Per Cent Dose		
0–1	1.4	2.0	0	0	15.7
1–2	1.4	0	0	0.8	1.5
2–3	0.9	2.1	0	1.9	0
3–4		0	0.8	2.2	0.2

* Experiment 13B, Subject T-3, I.V. Testosterone, 51 per cent excreted in 24 hours in urine; 10 per cent in bile.

† $T_{\frac{1}{2}}^{2}$ describes the rapid component in terms of "half-life" and $T_{\frac{1}{2}}^{1}$, the slow component, of the rate of excretion of hormone metabolites.

activity in the urine are also shown in this table. These terms "free" and "conjugated" refer to steroid hormones or metabolites easily extracted from an aqueous medium by a relatively non-polar solvent such as chloroform, methylene chloride and the like ("free") and to steroid derivatives which remain in the aqueous phase ("conjugated"). It is recognized that these terms are not completely accurate in the strict chemical sense since the products have not been isolated and characterized; nevertheless the designations have met with rather general acceptance and are convenient for a rough, general categorization. Over 90 per cent of labelled material was present as a conjugate both in the early and later portions of the first 24-hour urine collection. With the exception of Experiment 15, a maximum of 5 per cent of the radioactivity derived from any of the steroid hormones studied was excreted in the feces within four days.

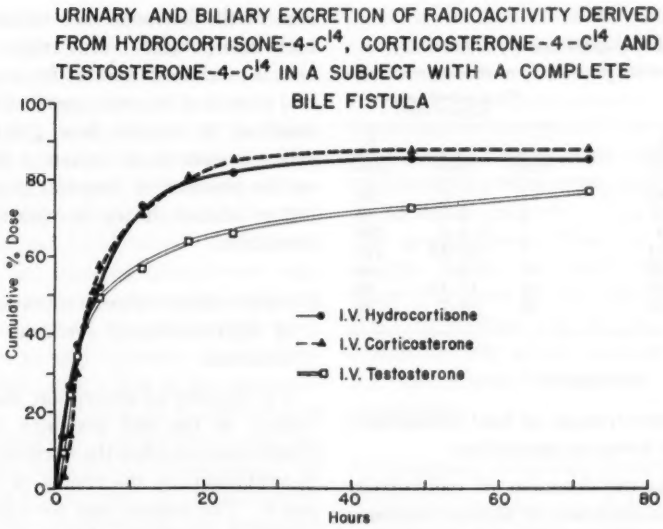

URINARY AND BILIARY EXCRETION OF RADIOACTIVITY DERIVED FROM HYDROCORTISONE-4-C14, CORTICOSTERONE-4-C14 AND TESTOSTERONE-4-C14 IN A SUBJECT WITH A COMPLETE BILE FISTULA
Cumulative % Dose
Hours
I.V. Hydrocortisone
I.V. Corticosterone
I.V. Testosterone

FIG. 4. URINARY AND BILIARY EXCRETION OF RADIOACTIVITY DERIVED FROM HYDROCORTISONE-4-C^{14}, CORTICOSTERONE-4-C^{14} AND TESTOSTERONE-4-C^{14} IN A SUBJECT WITH A COMPLETE BILE FISTULA

The relatively minor quantities of radioactivity excreted in bile are noted in Table IV.

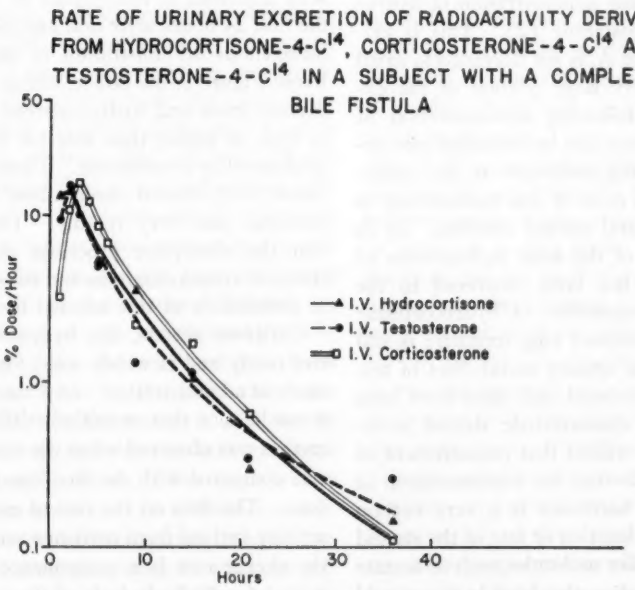

RATE OF URINARY EXCRETION OF RADIOACTIVITY DERIVED FROM HYDROCORTISONE-4-C14, CORTICOSTERONE-4-C14 AND TESTOSTERONE-4-C14 IN A SUBJECT WITH A COMPLETE BILE FISTULA
% Dose/Hour
Hours
I.V. Hydrocortisone
I.V. Testosterone
I.V. Corticosterone

FIG. 5. RATE OF URINARY EXCRETION OF RADIOACTIVITY DERIVED FROM HYDROCORTISONE-4-C^{14}, CORTICOSTERONE-4-C^{14} AND TESTOSTERONE-4-C^{14} IN A SUBJECT WITH A COMPLETE BILE FISTULA

TABLE V

Blood levels and conjugated urinary radioactivity

Blood levels			"Conjugated" urinary radioactivity		
Experiment	Time: *min.*	% Dose/Liter	Experiment	Time: *hours*	% Conjugated
4	40	0.82	3	1–2	94
	120	0.0	4	1–1.5	95
	210	0.0	6	1–2	100
7A	45	2.63		13–18	100
11	38	1.29	11	1–2	94
12	45	2.07	14	13–18	98
13A	45	1.84	15	1–2	96

DISCUSSION

Significance of measurements of total radioactivity for the study of hormone metabolism

In these studies the detection of radioactivity following the administration of steroid hormones labelled with radiocarbon at C-4 is interpreted as evidence for the presence either of the hormone or *in vivo* chemical transformation products which still possess the steroid ring structure. This interpretation is supported by three independent types of evidence. 1) To all practical purposes none of the C^{14} at carbon-4 is found in the expired carbon dioxide after the administration of hydrocortisone (6) or testosterone (2). This is evidence that at least Ring A, is not degraded to small fragments. 2) A very large portion of the excreted radioactivity following administration of either of these hormones can be extracted into organic solvents following hydrolysis of the conjugated metabolites and most of this radioactivity is recovered in the neutral steroid fraction. 3) A considerable portion of the total radioactivity of the neutral fraction has been recovered in the form of identified metabolites of hydrocortisone (8) in which the condensed ring structure is still intact. Almost all the urinary metabolites of testosterone have been isolated and these have been shown to retain the characteristic steroid structure (1). It is thus evident that measurement of total radioactivity following the administration of ring labelled steroid hormones is a very certain means for tracing the location or fate of the steroid nucleus. Unlike smaller molecules such as acetate or amino acids, the radioactive label in the steroid is not redistributed in the body and does not appear in compounds other than the direct metabolites of the hormone into which the isotope has been incorporated. This tracer technique, therefore, is much more specific for the study of the general aspects of hormone metabolism than any color reaction, no matter how great "specificity" is claimed, since in all instances these tests depend on the presence of chemical groupings which are lost or altered during *in vivo* biochemical transformation.

Comparison of oral and intravenous administration of hydrocortisone, cortisone acetate and testosterone

The rapidity of absorption, metabolism and excretion of the end products of hydrocortisone transformation when the hormone was given orally is established by the results of experiments 2, 3, and 4. The patient used for experiments 2 and 3 exhibited essentially the same excretory pattern in experiment 1 when the hormone was given intravenously and the latter result was in excellent agreement with the report previously published from these laboratories. From Figures 1 and 2, as well as Table II, it is clear that approximately as much radioactivity derived from hydrocortisone appeared as metabolites in the urine during the first 24 hours after oral administration as after intravenous administration in the same subject. From Figure 2, the rate at which the radioactivity derived from oral hydrocortisone is excreted was as high or higher than that for intravenously administered hydrocortisone. These results are consistent with clinical observations that oral hydrocortisone acts very rapidly. They also indicate that the absorptive processes do not delay the chemical transformations and subsequent excretion of metabolites of this adrenal hormone.

Cortisone acetate, like hydrocortisone, is effective orally and is widely used for therapy by this mode of administration. In Table II and Figure 2 it can be seen that an initially different rate of excretion was observed when the acetate of cortisone was compared with the free hormone, hydrocortisone. The data on the rate of excretion of radioactivity derived from cortisone acetate suggest that the slower rate is a consequence of the time required for the hydrolysis of the acetate ester with the release of free cortisone in the body. As hydrolysis progresses, free cortisone becomes avail-

able for biochemical conversion and the subsequent rate of excretion of radioactive metabolites from this material seems to be quite similar to that found with hydrocortisone.

It was hoped that an examination of the mode of metabolism and excretion would indicate some reason for the relative lack of activity of oral testosterone as compared with the adrenal hormones. The data indicate that 50 to 60 per cent of testosterone-4-C^{14} given intravenously to male subjects is excreted in urine as metabolites within 24 hours. Since testosterone has pharmacodynamic activity in females as well as males, it was of interest to obtain data on the fate of the male hormone in women. From the data it appears that testosterone is absorbed, transformed and excreted in a similar fashion by both males and females when given either orally or intravenously. In these and other experiments, the somewhat lower recovery of radioactivity within 24 hours after testosterone as compared with hydrocortisone seems to be a constant feature of the metabolism of the androgen.

Since two routes, the portal circulation and the lymphatics, can function in absorption from the intestine, the possibility existed that testosterone and hydrocortisone were absorbed by alternative paths. Cholesterol, a precursor of the steroid hormones with a similar structure, is largely absorbed through the lymphatics in man (9) and rodents (10). Kass, Hechter, Caspi, and Hycock (11) have given evidence that cortisone did not appear in the pleural fluid of a patient with chylothorax. In order to determine directly the route of absorption, a catheter was placed in the left thoracic duct of patients and the intestinal lymph was collected. When testosterone-4-C^{14} and hydrocortisone-4-C^{14} were separately given by mouth, radioactivity appeared in the urine and was almost completely absent from lymph. The only conclusion permitted from these data is that the absorption of both testosterone and hydrocortisone occurs via the portal system rather than by way of the intestinal lymphatics. In addition to establishing the route of absorption from the gastrointestinal tract of these hormones in man, the experiments also indicate that the difference in the biological effect of testosterone and hydrocortisone after oral administration must result from other causes than the route of absorption. Since most substances undergo some chemical change such as phosphorylation, esterification or the like during the absorptive process, it is quite possible that an initial alteration by the mucosa may result in loss of activity for one hormone while the biological activity of another may be unchanged or perhaps even enhanced. This is a subject for future investigation. However, if some chemical changes during the absorptive process are not found to account for the differences in activity of oral testosterone and hydrocortisone, reactions within the liver which inactivate testosterone, but not hydrocortisone must be advanced as an explanation.

Enterohepatic circulation of steroid hormones

Having demonstrated that orally administered testosterone and hydrocortisone directly enter the human liver via the portal circulation and in view of animal evidence which indicates that intravenously administered cortisone largely localized in the liver within a few minutes (3), a study of the possible enterohepatic circulation of these hormones or their metabolic products was undertaken in three subjects with complete bile fistulas. A considerable fraction of steroid hormone radioactivity is excreted in the bile by rodents and subsequently, in the rat appears in the feces (2, 3, 12), while the guinea pig (13) excretes this material in the urine. Man excretes the major portion of hormone metabolites in the urine. However, in the rat, when the bile duct is ligated the major portion of the derivatives of the hormone is excreted in the urine. The passage of the hormone and its metabolites through the bile and hence into the intestine is clearly an obligatory stage for the fecal excretion of hormone metabolites by the rat. It was anticipated therefore, that in man the hormone and its products either would not appear in the bile, or might be found in a form that could be readily reabsorbed from the intestine and subsequently excreted in the urine.

When hydrocortisone-4-C^{14} was administered intravenously at dose levels of 0.25 mg. and 100 mg. in two separate experiments in a patient with a complete bile fistula, direct measurement of the bile failed to disclose any significant amount of radioactivity. The total amount of radioactivity

that appeared in the urine within 24 hours was approximately the same as in an intact patient. Small quantities of hormone radioactivity, quite comparable with those of intact patients, appeared in the feces. Since similar results were obtained with the large and small doses, it is apparent that even a gross overload of hormone failed to alter the pattern by which the liver disposed of the steroid. This is clear evidence that the human species differs markedly from the rodent insofar as biliary excretion of hydrocortisone metabolites is concerned.

Radioactive testosterone was administered to a female patient with a complete bile fistula and 3 per cent of the radioactivity appeared in the bile. This was confirmed in a male subject, in whom approximately 10 per cent appeared in the bile within the first day while 51 per cent was recovered in the urine. It seems apparent from the studies on these two patients that a somewhat larger fraction of the metabolites of testosterone than of hydrocortisone appears in bile although the total excretion in this fluid is small when compared with the urinary output.

The absence of an enterohepatic circulation of hydrocortisone or its metabolites in man and the minor biliary excretion of testosterone metabolites have considerable significance for the interpretation of many studies dealing with the transformation products of these hormones and related derivatives. Thus, the possibility of metabolism of the hormones by the microorganisms of the gut can be disregarded and it can be considered established that the hormone metabolites in human urine are truly products formed by the various tissues that metabolize these compounds. The reverse of this situation is characteristic for the rodent. In the mouse, rat and guinea pig the existence of an actively operative enterohepatic circulation has been clearly defined (2, 3, 12, 13). This is particularly pertinent to studies of hormone metabolites by the guinea pig which superficially resembles man in that a major portion of the metabolites are found in the urine. Wyngaarden, Peterson, and Wolff (13) have shown, however, that most of these products were initially excreted into the gastrointestinal tract and reabsorbed before their final elimination in the urine. The role of the intestinal microorganisms in the transformations found in this species must, therefore, be kept clearly in mind, especially when comparison with the human is attempted.

The species difference in biliary elimination is very probably a consequence of a different type of conjugate formed by man and the rodents thus far studied. Evidence for this view can be found in the experiments with both testosterone and hydrocortisone. It was reported from this laboratory (2) that the urinary metabolites of testosterone in mice were for the most part persistently water-soluble products and that only a small fraction of these could be obtained in an ether soluble form even after drastic hydrolysis. A similar result has more recently been described for the biliary as well as the urinary metabolites of hydrocortisone (13) and cortisone acetate (12) in rats. Here, too, relatively little of the conjugated urinary steroids was cleaved by β-glucuronidase and even after acid hydrolysis about half the total radioactivity remained in the aqueous phase. The concordant results strongly suggest that dissimilar metabolites of these two hormones as well as conjugates other than glucuronic acid derivatives were formed. While the few metabolites that have been isolated from rodent urine are not very different from the human products these represented but a small portion of the compounds administered. There is thus reason to suggest that the difficulty in hydrolysis is associated in part with the nature of the non-steroidal portion of the complex as well as with the metabolites formed.

Comparison of the fate of corticosterone and hydrocortisone

The principal secretion of the adrenal cortex in the rodent is corticosterone whereas the principal secretion of the adrenal cortex in the human is hydrocortisone (4). The possibility, therefore, existed that if hydrocortisone were administered to a rodent or conversely if corticosterone were given to the human, these compounds might be treated as substances "foreign" to the species in which they were not the predominant adrenal secretion. This "foreign" nature of the hormone, rather than an intrinsic difference in its metabolism might account for the divergent results in the two species. Similar considerations might apply to cortisone which may also be a hormone "foreign" to the human species. Accordingly, corticosterone

was given to the same patient with the biliary fistula who had received the testosterone and hydrocortisone, as well as to another subject (B-1) with a complete biliary fistula. Corticosterone was metabolized, conjugated and excreted in amount and time very similar to that found for hydrocortisone. Sandberg, Migeon, and Samuels (14) have briefly reported a significant excretion in human bile of radioactivity derived from corticosterone; it is not possible at present to reconcile their findings with the results obtained in this study except, perhaps, that the clinical status of the patients, particularly liver function, may have been the differentiating factor.

In patient B-1, experiment 15, after corticosterone injection it is of particular interest to note that a relatively large amount of radioactivity, approximately 16 per cent, appeared in the feces within one day. Since there was no question about either the completeness of the biliary drainage or the absence of radioactivity in the bile, this radioactivity either entered the feces through the intestine or may have been the result of a chance radioactive contamination either on the ward or in the laboratory or is a finding related to the particular patient's clinical condition. The finding is different from the results in all other patients and an explanation cannot be offered for the fact at this time.

From the results obtained with all these steroid hormones there is no evidence for an effective enterohepatic circulation of these compounds or their metabolites in man.

Concluding remarks

The rates of excretion of conjugated radioactive metabolites for several hormones (Figures 2, 3, and 5) are quite similar, despite the fact that the total material recovered is somewhat different for each. A mechanism which may account for this similarity is one in which the kidney clears the conjugated hormone metabolites from a body compartment of constant but unknown size. This implies that the sum of the metabolic processes preceding the entrance of the transformed hormones into this particular physiologic space are both equivalent and rapid in all subjects. It may be presumed that the altered and conjugated hormone is in a space which, at a minimum, is the size of the extracellular fluid and may approach that of the total body water. The urinary steroids, which are almost exclusively in the form of conjugates, must be derived from this body compartment. The rate of excretion of the conjugated metabolites of these hormones would depend on the constant factors of the volume of the body compartment and clearance by the kidneys; the much more rapid rates which describe the immediate transformation of the hormone would not be apparent in the rate curve of excretion.

The data indicate that the liver occupies a central role in the metabolism of the steroid hormones and that the initiation of the unique biochemical processes which characterize the physiological activities of the hormones and the metabolic transformation of the steroid itself, occur within a very brief period of time. These very rapid processes in which the hormone triggers biochemical reactions in the body and either simultaneously or within a short time undergoes chemical transformation, are obscured in the gross excretion of the hormone and its metabolites. These studies, then, serve to emphasize the importance of identification of the various chemical transformation products of hormones as well as the necessity for more intensive study of the reactions of the hormones within a few minutes after administration. Perhaps by these means differences in hormone action as well as the etiological relationships of chemical disorders in hormone metabolism to disease can be brought into a coherent scheme along with the other information available on the problem of hormones. Some data concerned with the chemical nature and the amount of certain classes of hormone metabolites which constitute the "steroid pattern" have already been reported (15, 16); extension of these and related studies should yield important information.

SUMMARY

1. The absorption, distribution, biochemical alteration and excretion of steroid hormone metabolites have been studied in man following the oral or parenteral administration of labelled hydrocortisone, cortisone acetate, corticosterone, and testosterone.

2. In studies on subjects in whom the thoracic duct was cannulated, it was established that the

portal system is the route through which orally administered hydrocortisone and testosterone are absorbed.

3. There was no appreciable excretion of metabolites of these steroid hormones in the bile of patients with complete biliary fistulas. The absence of an enterohepatic circulation in the human represents a significant species difference when compared with the rodent where biliary excretion is obligatory.

4. Labelled steroid metabolites appear in the urine in the same quantities and at similar rates following oral or intravenous administration of hydrocortisone.

5. The lack of biological effect following the oral administration of testosterone compared with the parenteral route was shown to be unrelated to the rate or extent of absorption since the excretory pattern was the same in male and female subjects following either oral or intravenous labelled testosterone.

6. In spite of the extremely rapid excretion of the major portion of the steroid hormones studied, it was shown unequivocally that virtually all of the excretory products represented chemically altered metabolites of the original hormone.

7. These studies indicate that the reactions in which the hormone participates in order to exert its unique physiological activity occur within a short time following either the secretion or introduction of the steroid into the body.

ACKNOWLEDGMENTS

The authors are pleased to acknowledge the technical assistance of Ursel Blumenheim, Chrysie Costantakos, Hilda Denman, Ruth Loevinger and Julie Chen.

REFERENCES

1. Gallagher, T. F., Bradlow, H. L., Fukushima, D. K., Beer, C. T., Kritchevsky, T. H., Stokem, M., Eidinoff, M. L., Hellman, L., and Dobriner, K., Studies of the metabolites of isotopic steroid hormones in man. Recent Progress in Hormone Research, 1954, **9**, 411.
2. Barry, M. C., Eidinoff, M. L., Dobriner, K., and Gallagher, T. F., The fate of C^{14}-testosterone and C^{14}-progesterone in mice and rats. Endocrinology, 1952, **50**, 587.
3. Bradlow, H. L., Dobriner, K., and Gallagher, T. F., The fate of cortisone-*t* in mice. Endocrinology, 1954, **54**, 343.
4. Bush, I. E., The paper chromatography of steroids and its application to assay problems. Ciba Foundation Colloquia on Endocrinol., 1953, **5**, 203.
5. Bierman, H. R., Byron, R. L., Jr., Kelly, K. H., Gilfillan, R. S., White, L. P., Freeman, N. E., and Petrakis, N. L., The characteristics of thoracic duct lymph in man. J. Clin. Invest., 1953, **32**, 637.
6. Hellman, L., Bradlow, H. L., Adesman, J., Fukushima, D. K., Kulp, J. L., and Gallagher, T. F., The fate of hydrocortisone-4-C^{14} in man. J. Clin. Invest., 1954, **33**, 1106.
7. de Courcy, C., Bush, I. E., Gray, C. H., and Lunnon, J. B., A chromatographic investigation of Δ^4-3-ketosteroids and α-ketolic steroids in human urine. J. Endocrinol., 1953, **9**, 401.
8. Fukushima, D. K., Leeds, N. S., Bradlow, H. L., Kritchevsky, T. H., Stokem, M. B., and Gallagher, T. F., The characterization of four new metabolites of adrenocortical hormones. J. Biol. Chem., 1955, **212**, 449.
9. Hellman, L., Rosenfeld, R. S., and Gallagher, T. F., Unpublished data from this laboratory.
10. Chaikoff, I. L., Bloom, B., Siperstein, M. D., Kiyasu, J. Y., Reinhardt, W. O., Dauben, W. G., and Eastham, J. F., C^{14}-cholesterol. I. Lymphatic transport of absorbed cholesterol-4-C^{14}. J. Biol. Chem., 1952, **194**, 407.
11. Kass, E. H., Hechter, O., Caspi, E. Y., and Hycock, R. R., The route of absorption of orally administered cortisone in a patient with chylothorax. J. Clin. Endocrinol. & Metab., 1955, **15**, 167.
12. Bocklage, B. C., Doisy, E. A., Jr., Elliot, W. H., and Doisy, E. A., Absorption and metabolism of cortisone-4-C^{14} acetate. J. Biol. Chem., 1955, **212**, 935.
13. Wyngaarden, J. B., Peterson, R. E., and Wolff, A. R., Physiologic disposition of radio metabolites of hydrocortisone-4-C^{14} in the rat and guinea pig. J. Biol. Chem., 1955, **212**, 963.
14. Sandberg, A. A., Migeon, C., and Samuels, L. T., Distribution of radioactive hydrocortisone (compound F) and radioactive corticosterone (compound B) in human subjects. J. Clin. Endocrinol. & Metab., 1955, **15**, 851.
15. Kappas, A., Dobriner, K., and Gallagher, T. F., Studies in steroid metabolism. XXVII. A comparison of the steroid response to ACTH and cortisone in normal young men. J. Clin. Invest., 1955, **34**, 1559.
16. Kappas, A., and Gallagher, T. F., Studies in steroid metabolism. XXVIII. The α-ketosteroid excretion pattern in normal females and the response to ACTH. J. Clin. Invest., 1955, **34**, 1566.

THE EFFECTS OF COMPOUND A (11-DEHYDROCORTICOSTERONE) AND COMPOUND F (17-HYDROXYCORTICOSTERONE) ON TOTAL BODY FAT [1]

By LAURENCE H. KYLE, RICHARD J. MEYER,[2] MARCUS SCHAAF,[3] AND EDWARD J. WERDEIN

(From the Department of Medicine, Georgetown University Medical Center, Washington, D. C.)

(Submitted for publication April 4, 1956; accepted May 17, 1956)

Speculation regarding the influence of adrenal cortical hormones on body fat has engendered little experimental scrutiny, a deficiency which undoubtedly stems from the difficulties in performing *in vivo* measurement of the fat content of the body. Fundamental observations of Behnke, Feen, and Welham (1) on the relationship of body fat to body specific gravity suggested the possibility of utilizing serial measurements of body specific gravity for the evaluation of hormonally induced changes in body composition (2). Measurement of specific gravity by underwater weighing has been used in this laboratory to study the effects of ACTH, Compound E, Compound F and, to very limited degree, of Compound A on body fat (3). Because of an apparent increase in fat during treatment with Compound F, and because Compound A has been implicated as a fat-affecting hormone, more complete study of these hormones was believed desirable.

METHODS AND MATERIALS

The subject of this study was a 37-year-old white man with chronic dermatitis. He was hospitalized on the metabolic ward for two periods of about 4 months each under conditions of constant activity. The first study was performed when he was moderately depleted and the second study was conducted 15 months later after considerable repletion of fat and protein had been accomplished.

Body weight was measured each morning before breakfast, following completion of the 24-hour urine collection. The patient was given a constant diet containing 2200 calories, 90 grams of protein, 105 grams of fat and 225 grams of carbohydrate. Intake of sodium and potassium was calculated from standard food tables, allowance being made for sodium given as sodium thiocyanate. Rejected portions of the diet were given with the following meal; unfinished portions of the evening meal were given before sleep. The subject was permitted to leave the hospital for a few hours once weekly; there occurred only one major and a single minor violation of the dietary routine at these times.

Blood for laboratory analysis was drawn in relatively constant amounts and at regular intervals to provide uniform nitrogen loss by this route. Aliquots of 2-day pooled urine collections were refrigerated until analysis. Urinary nitrogen was measured by a micro-Kjeldahl technique. Nitrogen loss from blood sampling and in stools was estimated to total 1.6 gm. per day. Urinary sodium and potassium were measured by an internal standard flame photometer. Water balance was estimated from the difference between urine output and fluid intake, consisting of the liquid portion of the diet and measured amounts of distilled water.

Body specific gravity was obtained by the method of underwater weighing devised by Behnke, Feen, and Welham (1) as described from these laboratories (2, 3):

$$\text{Sp. Gr.} = \frac{\text{Weight in air}}{\text{Weight in air} - \text{true weight in water}}$$

Submerged weight was measured at a water temperature of 32 to 34° C. and corrected for residual lung volume, measured by the helium dilution technique of Willmon and Behnke (4). In correcting for residual lung volume, density of the water was ignored because of the minor error involved as well as the fact that data were obtained from successive measurements of the subject under the same standard conditions. At the time of each measurement underwater weight was obtained during both deep inspiration and full expiration. The difference was checked against measured vital capacity to insure expiration to a relatively constant volume of residual air at each weighing.

Total body fat was estimated by substitution in the Rathbun-Pace equation (5), formulated on the assumption that the specific gravity of fat is 0.918:

$$\% \text{ Fat} = 100\left(\frac{5.548}{\text{sp. gr.}} - 5.044\right)$$

Total body water was calculated from specific gravity by substitution in the equation derived by Osserman,

[1] This study was supported by the William Wade Hinshaw Fund and by a contract between Georgetown University and the Office of Naval Research. Reproduction in whole or in part is permitted for any purpose of the United States Government.

[2] Formerly a Research Fellow of the National Institutes of Health.

[3] Formerly the William Wade Hinshaw Cancer Research Fellow.

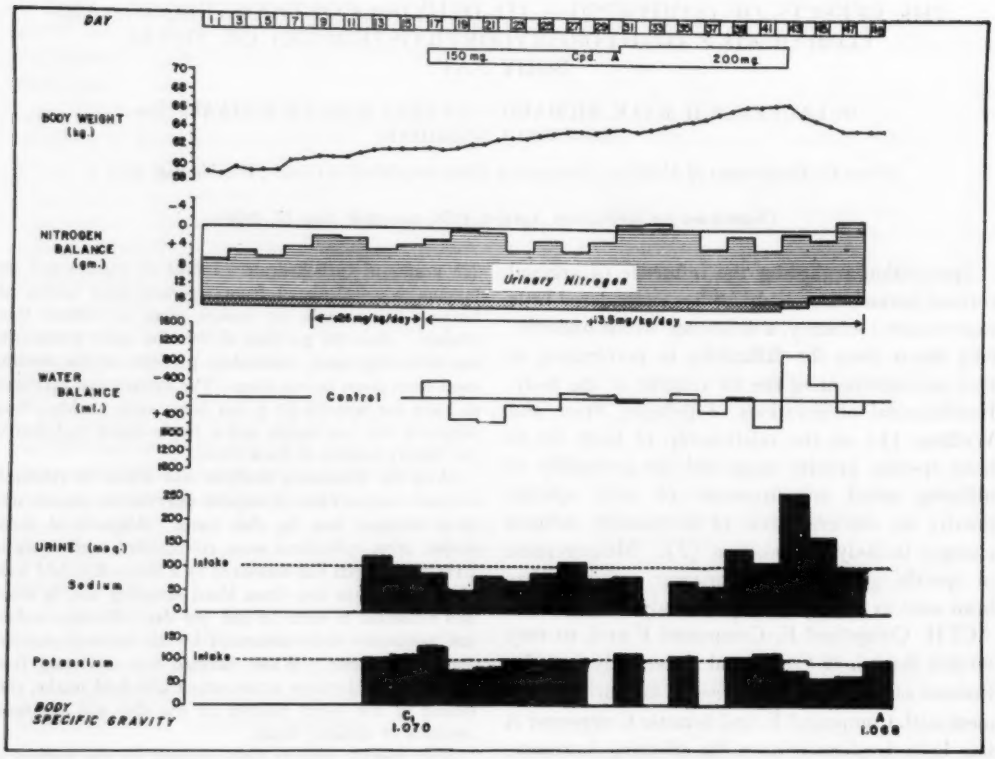


FIG. 1. METABOLIC CHANGES DURING TREATMENT WITH COMPOUND A

Pitts, Welham, and Behnke (6):

$$\% \text{ Water} = 100\left(4.340 - \frac{3.983}{\text{sp. gr.}}\right)$$

Fat free solids were assumed to comprise the difference between body weight and the sum of the weights of fat and water.

Because estimation of body composition by the specific gravity method is accurate only in the normally hydrated subject greatest reliance was placed on those measurements obtained when there existed the least amount of compartmental distortion. Parallel measurements of body water compartments were made by volume distribution techniques, particularly during the second study. Total body water (TBW) was estimated by measurement of antipyrine space, using the technique of Brodie, Axelrod, Soberman, and Levy (7) and by D_2O space, using the method of Broida, Morowitz, and Selgin (8). The extracellular fluid compartment (ECF) was assumed to be 70 per cent of the thiocyanate space, measured as described by Eder (9). Intracellular fluid (ICF) was assumed to be the difference between TBW and ECF. In all calculations involving total body water the values for antipyrine space were used because these checked much more closely with estimation of total body water by the specific gravity method than did the D_2O space.

Measurement of these fluid compartments permitted two methods of correction of hydration abnormality in estimating fat from body specific gravity. One consisted of substitution of the value for body density and that for antipyrine space in the equation derived by Siri (10):

$$\% \text{ Fat} = 225\ (1/D) - 0.76\% \text{ water} - 150.$$

Proper correction was made for the density of water (0.994 at the temperature used to obtain submerged weight) in conversion of specific gravity to density. The other correction applied was that of variation in ECF (corrected thiocyanate space) in the density equation: $D = \frac{\text{Mass.}}{\text{Volume}}$, as suggested by Keys and Brožek (11), correcting for the density of water on each occasion.

Calculation of change in body fat was also conducted by application of volume distribution techniques. The concept of the fat free body advanced by McCance and Widdowson (12) was utilized as follows:

$$\text{Fat} = \text{weight} - \text{T} - \left(\frac{\text{A} - \text{T}}{0.67}\right) - 0.075\left(\text{T} + \frac{\text{A} - \text{T}}{0.67}\right)$$

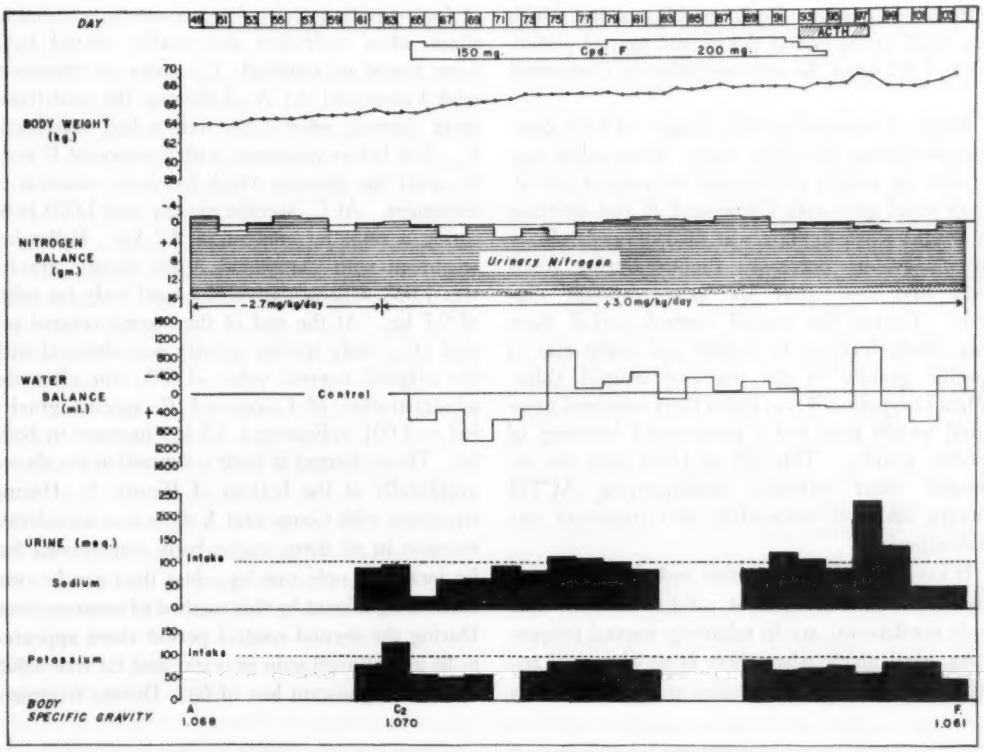


FIG. 2. METABOLIC CHANGES DURING TREATMENT WITH COMPOUND F

where T is corrected thiocyanate space and A is antipyrine space. Change in fat was also determined from the difference between total weight change and the sum of the changes in antipyrine space and nitrogen balance $\times 6.25$ (13).

Density of tissue gained was calculated from the ratio of change in the subject's weight to change in his volume.

Oxygen consumption was measured under fasting conditions by a closed circuit clinical apparatus. The average of two determinations was used in each instance. Urine calcium was measured by the method of Clark and Collip (14).

Compound A acetate was given intramuscularly in 3 to 4 divided doses each day. Compound F was administered orally every 6 to 8 hours.[4]

RESULTS

The metabolic changes which accompanied treatment with Compound A are shown in Figure 1. Nitrogen balance, which was very positive during the control period, became less positive, but only temporarily, when Compound A was started and when the dose was increased. Retention of both water and sodium occurred at these times. Body weight, which was increasing during the control period, rose more sharply during therapy but fell off quickly with the salt and water diuresis which followed cessation of treatment. Body specific gravity was 1.070 at the beginning of treatment —1.068 after the post-treatment diuresis.

The changes with Compound F are depicted in Figure 2. During the control period the patient was in approximate nitrogen balance and very little change resulted from the administration of Compound F. Retention of water and sodium was of similar magnitude to that noted with Compound A therapy. There was acceleration of weight gain, particularly when ACTH was given briefly at the termination of treatment. Cessation of all therapy was again followed by diuresis of salt and water

[4] These drugs were obtained through the generosity of Dr. Augustus Gibson of Merck & Co.

with loss of weight. Body specific gravity, which was 1.070 at the end of the second control period, fell to 1.061 after the administration of Compound F.

Figure 3 summarizes the changes in body composition during the entire study. Here again may be seen the weight gain during the control period, more rapid gain with Compound A and decrease in weight when therapy was discontinued. Body specific gravity decreased slightly during treatment and rose after the post-treatment diuresis. During the second control period there was steady increase in weight and slight rise in specific gravity to the previous control value. When Compound F was given there occurred more rapid weight gain and a pronounced lowering of specific gravity. This fell to 1.056 with the increased water retention accompanying ACTH therapy and rose moderately after treatment was discontinued.

It may be recalled that this technique of measurement is considered most valid when the major body constituents are in relatively normal proportions, particularly when there is no abnormal hydration. Consequently detailed analysis was made of four specific gravity determinations, selected at times when hydration abnormality should have been absent or minimal: C_1—prior to treatment with Compound A; A—following the post-treatment diuresis when body weight had stabilized; C_2—just before treatment with Compound F and; F—after the diuresis which followed cessation of treatment. At C_1 specific gravity was 1.070 indicating a total fat content of 8.7 kg. Following treatment with Compound A the specific gravity was 1.068, representative of a total body fat value of 9.7 kg. At the end of the second control period (C_2) body specific gravity was identical with the original control value—1.070; but after the administration of Compound F, specific gravity fell to 1.061 indicating a 3.5 kg. increase in body fat. These changes in body composition are shown graphically at the bottom of Figure 3. During treatment with Compound A there was a moderate increase in all three major body constituents but fat increased only one kg.—less than can be considered significant by this method of measurement. During the second control period there appeared to be a continued gain in water and fat free solids and an insignificant loss of fat. During treatment

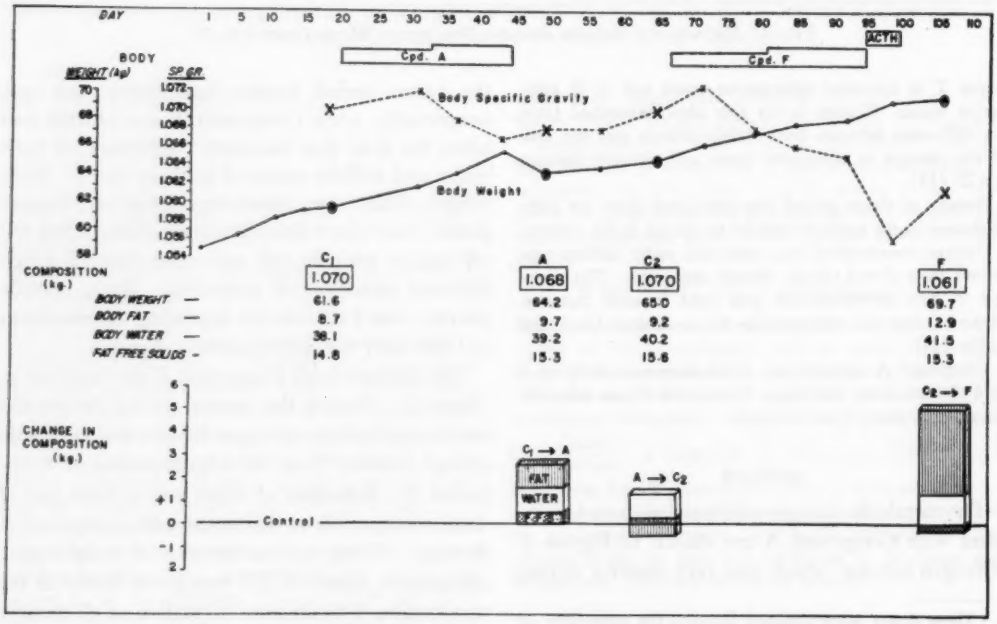


FIG. 3. CHANGES IN BODY COMPOSITION WITH COMPOUNDS A AND F

with Compound F a significant gain in fat is apparent.

These results indicated quite clearly that Compound A has no particular effect on body fat. There did appear to be a significant increase in total body fat during the administration of Compound F. Three possible explanations for these changes were considered:

1) Fat storage occurred only because protein repletion had become complete. Lack of a significant gain in fat during the second control period, when the patient was in approximate nitrogen balance, was a strong argument against this hypothesis.

2) True acceleration of fat deposition had occurred. This possibility, while more plausible than the first, lacked a clear explanation of the mechanisms involved. Insignificant protein catabolism and use of an isocaloric diet throughout the study indicated the absence of liponeogenesis and therefore some change in energy requirements or intermediary metabolism merited consideration.

3) The gain in fat was apparent rather than real because of change in other body constituents. Loss of material of high density, such as bone mineral, in sufficient amounts to cause such a significant change in specific gravity seemed very unlikely. The possibility of abnormal fluid retention could not be entirely discounted for antipyrine space determinations made throughout the study were too variable to permit precise correction for minor hydration abnormality. On the other hand the degree of water and sodium retention during therapy with Compound F appeared no greater than with Compound A, which demonstrated an insignificant effect on body specific gravity.

In view of these possibilities a second study was conducted, utilizing the same metabolic regimen but with the inclusion of multiple measurement of fluid compartments, so as to permit better correction of hydration abnormality and allow application of other methods of fat estimation. In addition, periodic measurement was made of oxygen consumption and urinary calcium excretion. During the 15 months' interval between studies the patient was maintained on 20 mg. of Compound F daily and encouraged to take more food so as to begin his second study at a higher level of body fat and to insure protein repletion. These objectives were accomplished, for at the beginning of the second study fat had increased to 13.5 kg.—nearly 5 kg. more than at the beginning of the first study—and protein stores were nearly replenished as indicated by the nitrogen balances.

Figure 4 depicts the metabolic changes and measurement of fluid compartments throughout the second study. Changes in weight were essentially the same as before, *i.e.*, steady slow gain during each control period, more rapid gain during treatment with Compound F and loss of weight after cessation of therapy. Despite the marked increase in excretion of sodium following the first course of Compound F no retention of sodium occurred during treatment; in fact urinary sodium exceeded that noted in the control period. During the second course of Compound F therapy there was moderate sodium retention and towards the end and after completion of treatment loss of sodium was noted. A moderate increase in nitrogen excretion accompanied the administration of larger doses of Compound F on one occasion but very little protein breakdown occurred during the second course of Compound F therapy. Total body water measurements gave no indication of overhydration; in fact, antipyrine and D_2O spaces indicated the existence of mild dehydration after the diuresis which followed cessation of treatment.

Figure 5 demonstrates the changes in body composition estimated from specific gravity measurements during the entire second study. During the first control period there was increase in weight without significant variation in specific gravity. Administration of Compound F was accompanied on both occasions by more precipitous gain in weight and significant lowering of specific gravity. The fall in weight after cessation of therapy was associated with but little return of specific gravity towards control levels.

As in the first study certain periods were selected for special analysis, chosen at times when minimal hydration abnormality was likely: C_1—at the beginning of study; C_2—immediately before the first course of Compound F; F_1—after the post-treatment diuresis; C_3—after a second control period and F_2—at the end of treatment.

The results of this study were generally in accord with the first. There occurred significant decrease in specific gravity indicative of substantial increase in fat during the administration of Compound F. Fat increased 1.9 kg. during F_1,

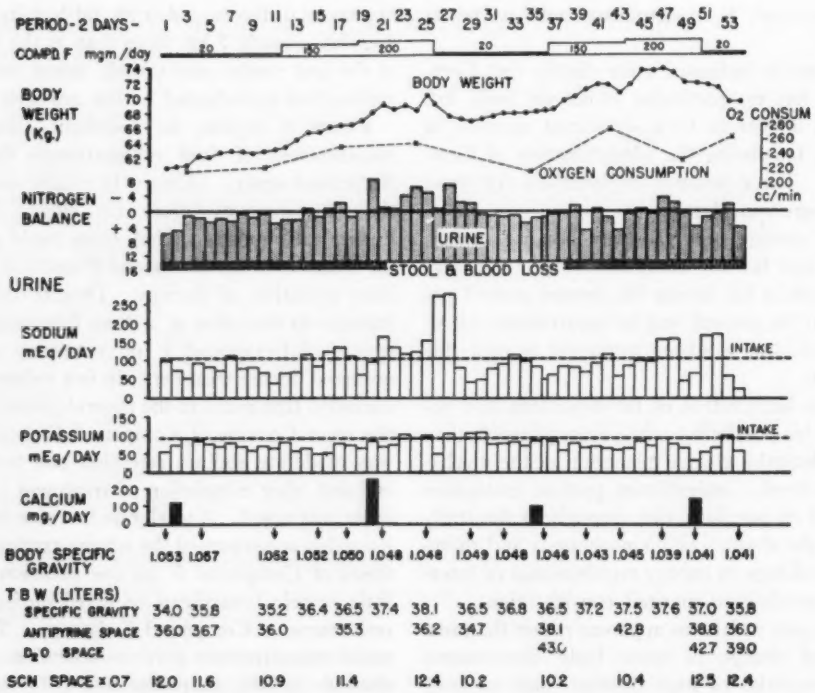


FIG. 4. CHANGES IN METABOLIC BALANCE AND FLUID COMPARTMENTS DURING TWO COURSES OF COMPOUND F

2.0 kg. during F_2 and totaled 5.3 kg. from C_2 to the end of the study. There also appeared to be a continued rapid gain in body fat between the two courses of Compound F. While this might suggest continued lipogenic effect of Compound F after cessation of treatment, it was more probably due to fluid reaccumulation. As shown by the determinations of total body water (Figure 4), there appeared to have been restoration of normal hydration during the second control period, which, because of addition of a material of low density, would indicate gain in fat. This apparent change in hydration suggests that gain of fat during treatment with Compound F was even more pronounced than indicated by the fat estimates derived from these specific gravity data.

As shown in Figure 4 oxygen consumption tended to increase during treatment with Compound F and excretion of calcium was insufficient to account for any measurable decrease in body specific gravity.

Table I compares the changes in body fat estimated by several different methods during the second study: (A) from specific gravity data (B) by substitution of body density and total body water (antipyrine space) in the same formula (C) by correction of the density equation for hydration abnormality as determined by change in thiocyanate space (D) by use of the technique of McCance and Widdowson (12) in estimation of the fat-free body mass and (E) by estimation of change in fat from change in body weight, change in total body water and nitrogen excretion. Use of these methods offers further support for the presence of a true increase in fat during the administration of Compound F. They also indicate that the specific gravity method of fat estimation is more uniform than those techniques which utilize only volume distribution. Although concurrent application of measurement of total body water to circumvent hydration abnormality has definite theoretical advantages it should be pointed out that the use of

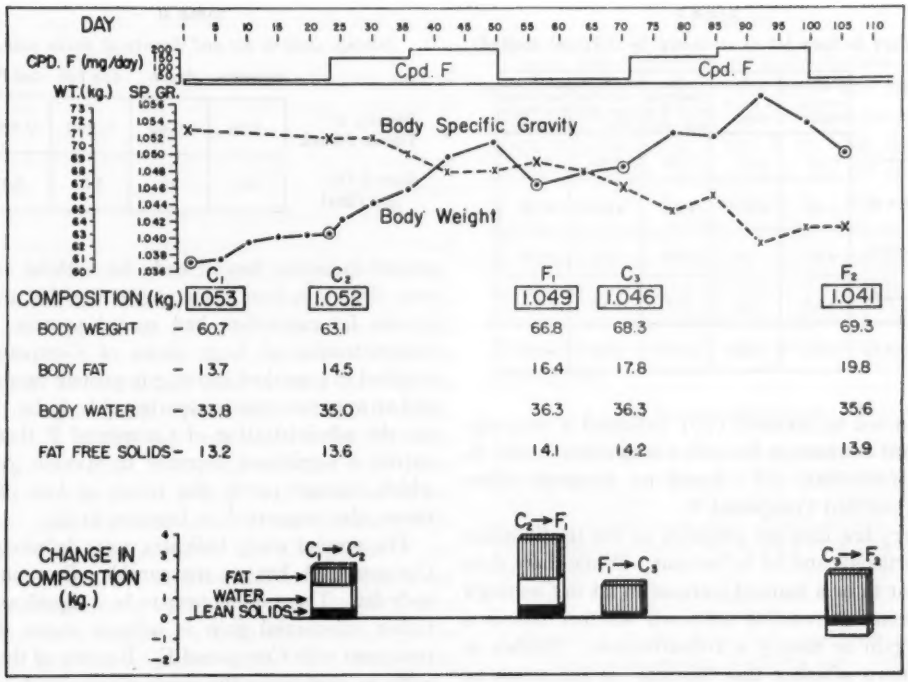


FIG. 5. CHANGES IN BODY COMPOSITION WITH COMPOUND F (SECOND STUDY)

poorly reproducible techniques, such as antipyrine space, cannot always strengthen the value of a readily reproducible method such as measurement of specific gravity.

Table II summarizes the results of both studies in terms of gain in fat per day and estimation of the density of the changed tissue as determined from measurements of body specific gravity. During the control period fat was gained at the rate of 41 gm. per day—during administration of Compound A fat gain averaged 32 gm. per day. When Compound F was given, fat increased at a rate of 90 gm. per day during the first study and 64 gm. per day during the second, the latter value being obtained from data which included the control period between the two courses of Compound F.

Calculation of density of added tissue revealed a value of 1.026 during the control period and 1.040 during treatment with Compound A indicating a gain of all major body constituents. When Compound F was administered density of added tissue was 0.940 during the first study and 0.941 during the second. Available data indicate that these values approximate those of adipose tissue (15, 16).[5]

DISCUSSION

The belief that adrenal hormones promote an increase in fatty tissue arose from clinical observation of the abnormal body configuration in Cushing's syndrome. Patients with this disorder usually manifest an apparent gain in fat, particularly distributed in the upper third of the body and frequently associated with wasting of the lower extremities. Studies of the relationship of adrenal hormones to body fat in lesser animals are so contradictory and difficult to interpret that complete review is redundant for the purpose of this discussion. Concise summaries of published work on the subject, such as those of Thorn and his associates (17), Ingle (18) and Engel and Scott (19) indicate that no clear-cut opinion regarding adrenal-fat relationships can be rendered. Studies

[5] It should be noted that adipose tissue consists of fat, water and protein and, consequently, has a greater density than pure fat.

TABLE I

Changes in body fat as estimated by different methods

	A SPECIFIC GRAVITY	B D + T&W (SIRI)	C CORRECTION OF D+$\frac{M}{V}$ BY SCN SPACE	D FROM FAT-FREE BODY MASS	E ANTIPYRINE & NITROGEN BALANCE
$C_1 \longrightarrow C_2$	+ 0.8	+ 1.7	+ 1.5	+ 1.8	+ 2.1
$C_2 \longrightarrow F_1$	+ 1.9	+ 3.8	+ 2.2	+ 5.4	+ 5.3
$F_1 \longrightarrow C_3$	+ 1.4	− 1.2	+ 1.4	− 3.9	− 1.9
$C_3 \longrightarrow F_2$	+ 2.0	+ 2.9	+ 1.7	+ 5.5	+ 3.0
$C_2 \longrightarrow F_2$	+ 5.3	+ 5.5	+ 5.3	+ 7.0	+ 6.4

TABLE II

Average gain in fat and density of tissue gained

	CONTROL	Cpd. A	Cpd. F (1)	Cpd. F (2)
Density of Tissue Gained	1.026	1.040	0.940	0.941
Gain in Fat (gm./day)	41	32	90	64

conducted by Kendall (20) indicated a very significant increase in fat with Compounds A and B, but Kochakian (21) found no lipogenic effect from purified Compound A.

Very few data are available on the relationships of corticoids and fat in humans. While there does appear to be a marked increase in fat in Cushing's syndrome it remains unknown whether this is a real gain or merely a redistribution. Neither is it known whether this "obesity" is the result of over-eating, the product of increased protein catabolism or tangible evidence of a direct hormonal effect. In fact we cannot even be sure that this local accumulation represents normal adipose tissue. Measurement of body fat by the specific gravity technique before and after the surgical correction of Cushing's syndrome is now underway in this laboratory. Preliminary observations indicate that correction of adrenal hyperfunction results in a significant decrease in body fat but also suggest that excessive body water is a major abnormality in Cushing's syndrome. Kinsell and his associates (22), from a study of diabetic patients, concluded that adrenal steroids accelerate the formation of carbohydrate from fat. Ziff, Simson, and Bunim (23) measured antipyrine, bromide and chloride spaces during adrenal stimulation in five patients with rheumatic disorders. In two cases weight gain exceeded the increase in antipyrine space to such degree as to indicate significant gain in body fat. Using the specific gravity method of fat estimation Doolan, Welham, and Kyle (3) studied the effects of ACTH and adrenal steroids on total body fat. ACTH appeared to induce loss of body fat without significant change in lean tissue suggesting that the excessive fat catabolism had spared protein. The administration of large doses of Compound E resulted in a marked increase in protein catabolism and an apparent relative sparing of body fat. During the administration of Compound F there occurred a significant decrease in specific gravity, which, though partly the result of loss of lean tissue, also suggested an increase in fat.

The present study indicates quite definitely that Compound A has no measurable effect on total body fat. There did appear to be a significant and rather substantial gain in adipose tissue during treatment with Compound F. Because of the difficulty in explaining the mechanisms involved and in view of the limited application of these body compositional measurements in clinical investigation, it seems pertinent to re-evaluate the methodology employed before assuming that the gain in fat was real.

As pointed out by Keys and Brožek (11) the specific gravity of the fat free human body cannot be determined with precision. Furthermore, the fat prediction equation utilized in these studies is derived from measurement of small animals, whose proportional body composition and skeletal mass differs from the human subject. It is therefore obvious that a single determination of specific gravity, or even multiple determinations in a metabolically stable subject, cannot provide data which will portray the exact amount of body fat. In conducting serial determinations on a subject of changing fat content, however, a much higher degree of accuracy is possible. In our hands the summation of possible errors, including measurement of residual lung volume, accuracy of scale and reproducibility of replicate determinations indicate that loss or gain of fat must approach 1.5 kg. to be significant. The gains in fat

with Compound F observed here were well in excess of that figure.

Apparent gain in fat, as judged by lowering of specific gravity, could also result from addition of other material of low density, such as water, or loss of body constituents of high density, such as protein or minerals. In the study reported here it is obvious that there was insufficient loss of high density body constituents to account for more than a fraction of the specific gravity change. Consequently the existence of significant overhydration remains as the only factor which could lead to distortion of the data sufficient in degree to produce an artifactual gain in body fat. In the first study variation in total body water determinations was of sufficient magnitude to prohibit correction of minor hydration abnormality. However, the changes in weight, sodium excretion and water retention were nearly identical during treatment with Compound A and Compound F and only in the latter instance did there occur a significant change in specific gravity. In the second study more accurate measurement of antipyrine space and the addition of D_2O and thiocyanate space measurements provided a clear indication that no significant water retention occurred. In fact, there appeared to be mild dehydration after the diuresis which followed cessation of treatment with Compound F. Such loss of water would tend to minimize the measured gains in body fat which occurred during therapy. Consequently all evidence indicates that the gain in fat was truly significant during treatment with Compound F.

The mechanisms responsible for the increase in body fat are completely obscure. Liponeogenesis from protein obviously did not occur and, as judged by oxygen consumption, there was no decrease in energy production during therapy. Thus we can only postulate some change in intermediary metabolism which permitted the addition of fat without appreciable change in the measured components of the metabolic pool. This supposition obviously lends itself to speculation regarding the hypothesis that some persons, at least under certain circumstances, can maintain their weight or even become obese on a remarkably small caloric intake. Studies of the type reported here should provide more exact data regarding gain or loss of body fat and permit a more objective approach to the problems of obesity.

SUMMARY

Serial determinations of body specific gravity were combined with balance study and measurement of body water compartments to determine the effect of Compounds A and F on body fat. The administration of Compound A for 26 days had no demonstrable effect on body fat. The administration of Compound F for similar periods of time on three separate occasions was associated with a significant increase of total body fat. This gain in fat was not accompanied by evidence of either liponeogenesis from protein or decreased energy production.

ACKNOWLEDGMENT

We would like to express our appreciation for the assistance of the personnel of the Experimental Diving Unit, U. S. Naval Gun Factory in performing these studies. The advice of Drs. Albert Behnke and Will Siri has been invaluable in the conduct of this investigation.

REFERENCES

1. Behnke, A. R., Jr., Feen, B. G., and Welham, W. C., The specific gravity of healthy men. Body weight ÷ volume as an index of obesity. J.A.M.A., 1942, **118**, 495.
2. Kyle, L. H., Welham, W. C., Doolan, P. D., and Schaaf, M., Study of the extrathyroidal effects of thyroid-stimulating hormone. J. Clin. Endocrinol. & Metab., 1954, **14**, 1029.
3. Doolan, P. D., Welham, W. C., and Kyle, L. H., Studies on the effect of ACTH and certain adrenal steroids on total body fat. Metabolism, 1955, **4**, 39.
4. Willmon, T. L., and Behnke, A. R., Residual lung volume determinations by the methods of helium substitution and volume expansion. Am. J. Physiol., 1948, **153**, 138.
5. Rathbun, E. N., and Pace, N., Studies on body composition. I. The determination of total body fat by means of the body specific gravity. J. Biol. Chem., 1945, **158**, 667.
6. Osserman, E. F., Pitts, G. C., Welham, W. C., and Behnke, A. R., In vivo measurement of body fat and body water in a group of normal men. J. Applied Physiol., 1950, **2**, 633.
7. Brodie, B. B., Axelrod, J., Soberman, R., and Levy, B. B., The estimation of antipyrine in biological materials. J. Biol. Chem., 1949, **179**, 25.
8. Broida, H. P., Morowitz, H. J., and Selgin, M., Optical spectroscopic determination of hydrogen isotopes in aqueous mixtures. J. Res. Nat. Bur. Stand., 1954, **52**, 293.
9. Eder, H. A., Determination of thiocyanate space. Methods in Med. Research, 1951, **4**, 48.

10. Siri, W., Personal communication.
11. Keys, A., and Brožek, J., Body fat in adult man. Physiol. Rev., 1953, **33**, 245.
12. McCance, R. A., and Widdowson, E. M., A method of breaking down the body weights of living persons into terms of extracellular fluid, cell mass and fat, and some applications of it to physiology and medicine. Proc. Roy. Soc., London, Series B, 1951, **138**, 115.
13. Moore, F. D., Haley, H. B., Bering, E. A., Jr., Brooks, L., and Edelman, I. S., Further observations on total body water. II. Changes of body composition in disease. Surg., Gynec. & Obst., 1952, **95**, 155.
14. Clark, E. P., and Collip, J. B., A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem., 1925, **63**, 461.
15. Behnke, A. R., Osserman, E. F., and Welham, W. C., Lean body mass. Its clinical significance and estimation from excess fat and total body water determinations. Arch. Int. Med., 1953, **91**, 585.
16. Keys, A., Anderson, J. T., and Brožek, J., Weight gain from simple overeating. I. Character of the tissue gained. Metabolism, 1955, **4**, 427.
17. Thorn, G. W., Jenkins, D., Laidlaw, J. C., Goetz, F. C., Dingman, J. F., Arons, W. L., Streeten, D. H. P., and McCracken, B. H., Pharmacologic aspects of adrenocortical steroids and ACTH in man. (Continued.) New England J. Med., 1953, **248**, 284.
18. Ingle, D. J., The biologic properties of cortisone: A review. J. Clin. Endocrinol., 1950, **10**, 1312.
19. Engel, F. L., and Scott, J. L., The role of hormones in adipose tissue glycogen synthesis in the rat: The adrenal cortex. Endocrinology, 1951, **48**, 56.
20. Kendall, E. C., Influence of the adrenals on carbohydrate metabolism. Conf., on Metabolic Aspects of Convalescence Including Bone and Wound Healing, 10, 1945, 81.
21. Kochakian, C. D., Adrenal cortical steroids. Conf. on Metabolic Aspects of Convalescence Including Bone and Wound Healing, 6, 1944, 13.
22. Kinsell, L. W., Michaels, G. D., Margen, S., Partridge, J. W., Boling, L., and Balch, H. E., The case for cortical steroid hormone acceleration of neoglucogenesis from fat in diabetic subjects. A summary of five years' investigative work. J. Clin. Endocrinol. & Metab., 1954, **14**, 161.
23. Ziff, M., Simson, J., and Bunim, J. J., Effect of ACTH on water distribution in man as measured by antipyrine, T 1824 and bromide. J. Clin. Invest., 1952, **31**, 829.

PLASMA REGRESSION, DISTRIBUTION AND EXCRETION OF RADIOMERCURY IN RELATION TO DIURESIS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF Hg^{203} LABELLED CHLORMERODRIN TO THE DOG[1]

By R. R. M. BORGHGRAEF,[2] R. H. KESSLER,[3] and R. F. PITTS with the assistance of M. E. PARKS, W. VAN WOERT, and M. B. MacLEOD

(From The Department of Physiology, Cornell University Medical College, New York City, N. Y.)

(Submitted for publication April 2, 1956; accepted May 14, 1956)

Radioactive mercury, administered intravenously in the form of an organic mercurial diuretic, Meralluride (Mercuhydrin®), leaves the plasma of man as a multiple exponential function of time. Threefoot, Ray, Burch, Cronvich, Milnor, Overman, and Gordon (1) demonstrated that no less than three major exponential rates are responsible for this regression of plasma mercury concentration. They postulated that the fastest rate represents mechanical mixing in the plasma compartment; that the intermediate rate represents rapid adsorptive, chemical and diffusion phenomena, including migration into tissues; and that the slowest rate represents mainly renal excretion.

Early in our work with mercurial diuretics labelled with radioactive mercury, we observed that Chlormerodrin (Neohydrin®), administered in a single intravenous dose, disappears from the plasma of the dog in a similar fashion. Indeed the time constants of the individual exponentials in the dog were comparable to those in man. Experiments described below were undertaken to test the postulates of Threefoot and his co-workers (1) concerning the nature of the processes involved in the clearing of mercury from the plasma and have served to verify and amplify their conclusions. Additional experiments describe the extraction, excretion and accumulation of mercury by the kidneys and relate these processes to the development of diuresis and to the disappearance of mercury from the plasma.

[1] Aided by grants from the Life Insurance Medical Research Fund and the National Heart Institute of the National Institutes of Health.

[2] Fellow of the Belgian-American Educational Foundation.

[3] Fellow of the Life Insurance Medical Research Fund.

METHODS

Our experiments, some 34 in all, have been performed on female mongrel dogs lightly anesthetized with sodium pentobarbital. Arterial blood samples were drawn through an indwelling needle in the femoral artery. In certain experiments renal venous blood samples were drawn through a polyethylene catheter, introduced by way of the jugular vein and positioned in the right renal vein by manipulation through an abdominal incision. Urine samples were collected either through a Foley catheter, introduced into the bladder or through ureteral catheters, inserted by way of an abdominal incision.

The creatinine clearance has been employed as a measure of glomerular filtration rate and the creatinine clearance, divided by the plasma extraction ratio and corrected for urine flow (2, 3), has been employed as a measure of renal plasma flow. Chemical methods have been described in previous communications from this (4) and other (5) laboratories.

Chlormerodrin (3-chloromercuri-2-methoxy-propylurea, Neohydrin®) has been synthesized in our laboratory using radiomercury, Hg^{203}, according to directions supplied

TABLE I

An experiment on a normal dog illustrating the development of diuresis and the plasma regression, clearance and excretion of mercury following the intravenous administration of 1.0 mg. of mercury per kg. as Chlormerodrin

ELAPSED TIME	URINE FLOW	CREATININE CLEARANCE	SODIUM			CHLORMERODRIN MERCURY			
			PLASMA	EXCRETED	REABSORBED	PLASMA	UV/P	C_{Hg}/C_{Cr}	EXCRETED
min.	ml./min.	ml./min.	mEq./L.	mEq./min.	% filtered	ppm./ml.	ml./min.		% dose administered
−90	Infuse 0.85% NaCl, 0.40% Creatinine at 5 ml./min., intravenously								
−30–15	1.53	46.7	152	0.33	95.1	—	—	—	—
−15–0	2.20	46.5	152	0.42	93.8	—	—	—	—
0	in 20 sec. 16.4 mg. (1.0 mg./kg.) Hg as Chlormerodrin, intravenously								
0–5	2.60	47.5	151	0.51	92.6	10.9	0.8	0.02	0.3
5–10	2.40	48.5	150	0.49	93.0	6.55	17.7	0.37	3.8
10–20	3.10	51.7	151	0.65	91.2	4.83	21.1	0.41	10.0
20–30	3.80	49.6	150	0.70	90.1	3.74	20.7	0.42	14.7
30–45	5.93	51.4	151	1.12	84.9	3.04	21.0	0.41	20.6
45–60	8.33	57.5	149	1.52	81.4	2.44	24.2	0.42	26.0
60–75	9.60	55.7	150	1.70	78.7	2.10	24.0	0.43	30.6
75–90	8.53	55.7	149	1.53	80.6	1.78	25.8	0.45	34.8
90–105	8.40	58.9	149	1.51	81.9	1.48	30.1	0.51	38.9
105–120	7.73	54.2	148	1.40	81.6	1.21	33.0	0.61	42.5
120–140	7.90	58.0	149	1.37	83.3	1.10	32.5	0.56	46.9
140–160	6.75	54.0	148	1.14	85.0	0.83	35.7	0.66	50.5
160–180	6.55	55.4	147	1.08	86.0	0.71	38.3	0.69	55.0

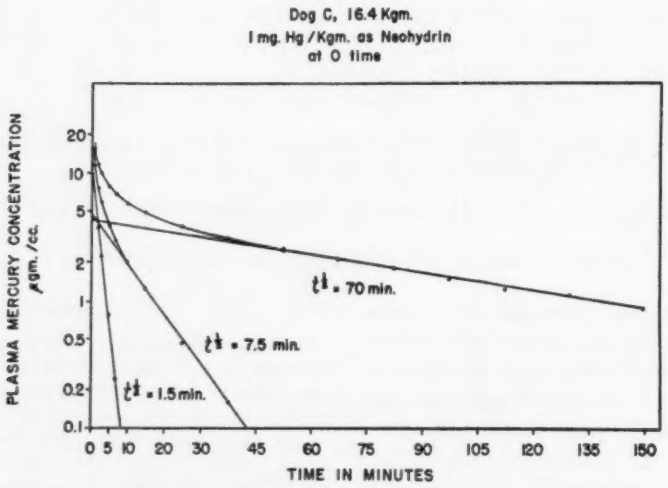


FIG. 1. AN ANALYSIS OF THE CURVE DESCRIBING THE REGRESSION OF PLASMA CONCENTRATION FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN

The upper original or mother curve is a semilogarithmic plot of data obtained in the experiment presented in Table I. The three straight lines representing the dominant exponentials making up the mother curve were derived graphically as described in Footnote 6.

by Dr. H. L. Friedman of the Lakeside Laboratories, Inc. In all experiments, 1.0 mg. of mercury as Chlormerodrin per kg. of body weight has been administered intravenously into a foreleg vein in 20 seconds. This dose is equivalent to the human therapeutic dose of most mercurial diuretics. Plasma and urine samples were counted without preparation other than dilution, in a well-type scintillation counter; sufficient counts were accumulated to provide an accuracy of 1 to 5 per cent.[4] In experiments in which the uptake of mercury by the kidney was measured directly, the left kidney was exteriorized in the flank and a shielded Geiger tube applied directly to its surface, separated only by a single layer of Saran wrap. A sheet of dental dam, perforated to permit passage of the renal pedicle, served to hold the kidney in close approximation to the Geiger tube.

RESULTS

In Table I are summarized data obtained in a representative experiment in which 1.0 mg. of mercury, Hg^{203}, per kg. body weight was administered as Chlormerodrin. Following two 15-minute control periods, the diuretic was injected rapidly into a foreleg vein in 20 seconds. It is evident that the diuresis began rather slowly, either within the 10 to 20-minute period or perhaps the 20 to 30-minute period following the injection. It reached its peak in terms of urine flow, sodium excretion and depression of sodium reabsorption during the 60 to 75-minute period, although for the full three-hour duration of the experiment depression of salt and water reabsorption was clearly evident.

During the first five minutes after injection of Chlormerodrin, the excretion of mercury was negligible. Thereafter, the clearance [5] of mercury rose to a level some 40 to 60 per cent of filtration rate, and by the end of three hours, 55 per cent of the

[4] Activity of Chlormerodrin has ranged between 4,000 and 800 counts per minute per microgram of mercury when counted in a well-type scintillation counter.

[5] The term clearance has certain unique connotations when applied to mercurial diuretics. Chlormerodrin is excreted in the urine as a cysteine or acetyl-cysteine complex. The former is presumably formed in the kidney in the process of tubular transport; the latter is formed in the liver (6). In the plasma it is in large part bound to SH groups of proteins. Thus the form of the compound in plasma differs from that in urine, a fact which necessitates the broadest possible interpretation of clearance. Of even more import is the fact that large amounts of the diuretic are stored in the tubules in the course of renal excretion. An undetermined time lag is introduced between removal of the drug from the blood plasma and its delivery into the urine.

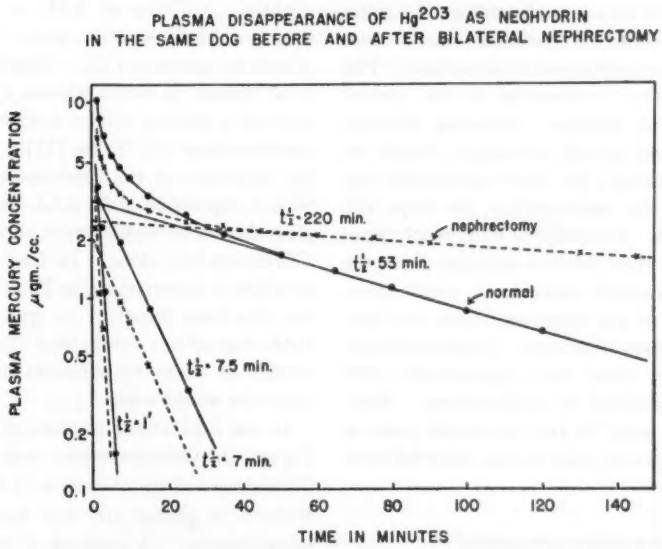


FIG. 2. A COMPARISON OF CURVES OF REGRESSION OF PLASMA CONCENTRATION FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN IN THE SAME DOG IN A CONTROL EXPERIMENT AND FOLLOWING BILATERAL NEPHRECTOMY

dose administered had been excreted in the urine. Comparable clearances and rates of mercury excretion have been previously noted in man (7, 8) following the administration of Meralluride. Such rapid rates of excretion are the more surprising in view of the high degree of binding of mercurial diuretics to plasma protein (9, 10). At the lower plasma mercury concentrations, one per cent or less of Chlormerodrin is filterable, hence free to enter the urine in the glomerular filtrate. Obviously tubular secretion must play an important role in the urinary excretion of this agent.

Figure 1 continues the analysis of the data presented in Table I. In the upper curve, the logarithm of the plasma concentration of mercury has been plotted as a function of time. This curve has been graphically analyzed into its three exponential components by the method of successive subtraction.[6] The processes which determine these exponentials have half-times of 70, 7.5 and 1.5 minutes,[7] respectively, values comparable to those observed for the processes involved in the removal of Meralluride from the plasma of man.

Origin of the slowest exponential

As was mentioned above, Threefoot and his associates (1) postulated that the slowest exponential is dominated by renal excretion of the diuretic. If so, bilateral nephrectomy should markedly alter the later time course of the plasma concentration curve. In Figure 2, the upper two curves describe the disappearance of mercury

[6] The upper or so-called mother curve is described by an equation of the following form: $P = Ae^{-at} + Be^{-bt} + Ce^{-ct}$, in which P is the plasma concentration at any time, t; a, b, and c are the slopes of the three straight lines on semi-logarithmic coordinates; and A, B and C are the extrapolated intercepts of these straight lines on the X axis. To analyze the mother curve into its exponential components, the best straight line is fitted by inspection to the points from 50 to 150 minutes. The differences between the mother curve and this straight line, extrapolated to the X axis, are plotted as the first daughter curve. This curve analyzed in the same fashion yields the third straight line. In essence, this means that at any moment at least three separate and independent groups of processes are operating to clear mercury from the plasma. Each group operates at a rate that is proportional at any moment to the existing plasma mercury concentration.

[7] In 14 similar experiments the half-times of these 3 exponentials have ranged between 50 and 80, 6 and 9, and 0.5 and 1.5 minutes, respectively.

from the plasma in two experiments on the same dog; the first, a control experiment; the second, immediately following bilateral nephrectomy. The half-time of the slow exponential in the control experiment was 53 minutes; following bilateral nephrectomy, it was greatly prolonged, namely to 220 minutes. Although the slow exponential was markedly altered by nephrectomy, its slope did not approach zero. Accordingly extrarenal clearance mechanisms must remove mercury from the plasma at an appreciable rate; hence renal excretory mechanisms do not alone determine the later time course of plasma clearance. Graphic analysis indicated that the other two exponentials were not appreciably modified by nephrectomy. Similar findings were noted in two additional pairs of experiments performed prior to and after bilateral nephrectomy.

Origin of the intermediate exponential

According to Threefoot and his associates (1), migration of mercury from the vascular compartment into the tissues is the major determinant of the intermediate exponential. Several lines of evidence indicate that BAL, dithiopropanol, increases the diffusibility of Chlormerodrin. Administration of BAL plus Chlormerodrin increases the mercury content of all tissues other than the kidney (11). Chlormerodrin enters red cells but slightly. Addition of BAL to whole blood containing Chlormerodrin causes rapid penetration of cells by mercury (12). Administration of BAL to an animal in which plasma Chlormerodrin has reached a plateau causes a sharp drop in plasma concentration (*cf.* Table III). It is evident that the half-time of the intermediate exponential of plasma regression of the BAL-Chlormerodrin complex should be significantly shorter than that for Chlormerodrin alone. In four experiments, one of which is summarized in Table III and Figure 3, this has been found to be true. However, since BAL has effects other than that on diffusibility, results of these experiments are more complex than one would wish.

In the experiment summarized in Table II and Figure 3, Chlormerodrin was complexed with dithiopropanol in saline in a 1 : 4 molar ratio, neutralized to phenol red and immediately injected intravenously. A dose of 2 ml. of 10 per cent BAL in oil was given intramuscularly 30 minutes before the start of the experiment. Excess BAL was deemed necessary because of its known rapid rate of oxidation.

It is evident from Table II that no true diuresis resulted from administration of the BAL-Chlor-

TABLE II

An experiment on a BAL treated dog illustrating the plasma regression, clearance and excretion of mercury following the intravenous administration of 1.0 mg. of mercury per kg. as the Chlormerodrin-BAL complex

ELAPSED TIME	URINE FLOW	CREATININE CLEARANCE	SODIUM			CHLORMERODRIN MERCURY			
			PLASMA	EXCRETED	REABSORBED	PLASMA	UV/P	C_{Hg}/C_{Cr}	EXCRETED
min.	ml./min.	ml./min.	mEq./L.	mEq./min.	% filtered	μgm./ml.	ml./min.		% dose
−150	Infuse 0.85% NaCl, 0.40% Creatinine at 5 ml./min., intravenously								
−60	2 ml. 10% BAL in oil, intramuscularly								
−30–15	2.27	51.2	144	0.15	98.0	–	–	–	–
−15–0	3.00	54.3	144	0.20	97.5	–	–	–	–
0	in 20 sec. 14.0 mg. (1.0 mg./kg.) Hg as Chlormerodrin–BAL complex, i.v.								
0–5	3.00	50.8	143	0.20	97.5	0.85	89.0	1.75	2.7
5–10	2.43	55.9	144	0.18	97.8	0.48	136.0	2.44	6.5
10–20	3.00	55.5	143	0.24	97.0	0.42	78.7	1.42	8.4
20–30	3.30	54.8	143	0.25	96.8	0.40	73.5	1.34	10.6
30–45	4.10	54.4	143	0.26	96.8	0.35	58.5	1.08	12.8
45–60	4.40	54.9	145	0.24	97.0	0.46	32.8	0.60	14.4
60–75	4.60	52.3	145	0.22	97.1	0.38	28.7	0.55	15.6
75–90	5.47	51.9	144	0.23	97.0	0.34	30.5	0.59	16.8
90–105	6.40	50.3	144	0.25	96.6	0.27	33.5	0.67	17.7
105–120	6.60	50.5	145	0.25	96.8	0.35	25.6	0.51	18.7
120–135	7.12	51.8	147	0.26	96.7	0.28	32.9	0.62	19.7
135–150	6.97	51.2	148	0.32	96.1	0.30	30.0	0.59	20.6

TABLE III

An experiment on a unilaterally nephrectomized dog illustrating the development of diuresis in relation to plasma regression, excretion and renal extraction and accumulation of mercury following the intravenous administration of 1.0 mg. of mercury per kg. as Chlormerodrin

ELAPSED TIME	URINE FLOW	GLOMERULAR FILTRATION RATE	RENAL PLASMA FLOW	CHLORMERODRIN MERCURY					
				ARTERIAL PLASMA	VENOUS PLASMA	EXTRACTION	EXCRETION	ACCUMULATION	UV/P
min.	ml./min.	ml./min.	ml./min.	μgm./ml.		μgm. cumulative			ml./min.
−150	Infuse 0.85% NaCl, 0.4% Creatinine at 5 ml./min., intravenously								
−30–15	1.7	26.1	111	–	–	–	–	–	–
−15–0	2.0	27.3	101	–	–	–	–	–	–
0	in 20 sec. 13.0 mg. (1.0 mg./kg.) Hg as Chlormerodrin, intravenously								
1	–	–	–	11.05	8.65	279	–	–	–
2	–	–	–	7.98	5.80	529	–	–	–
3	–	–	–	6.52	4.45	764	–	–	–
4	–	–	–	5.83	4.13	958	–	–	–
0–5	2.0	26.5	109	5.15	3.82	1112	230	882	6.4
5–10	2.2	22.9	90	4.60	3.29	1737	498	1239	11.7
10–20	5.35	30.3	145	3.30	2.59	2877	980	1897	14.6
20–30	5.50	31.0	180	2.73	2.23	3897	1319	2578	12.4
30–45	5.67	26.8	149	2.11	1.70	4959	1774	3185	14.3
45–60	6.00	24.8	105	1.70	1.32	5725	2187	3538	16.2
60–77	6.06	25.1	110	1.36	1.12	6273	2609	3664	18.2
77–90	5.85	24.9	108	1.175	0.965	6642	2917	3725	20.2
90–105	4.60	24.9	96	0.992	0.733	7067	3245	3822	22.1
105–120	3.80	22.6	75	0.869	0.604	7400	3517	3883	21.0
120	2 ml. 10% BAL in oil, intramuscularly								
120–135	1.33	13.7	37.2	0.176	0.290	7342	4025	3317	192.0
135–150	0.20	19.6	62.8	0.175	0.398	7144	5395	1749	522.0

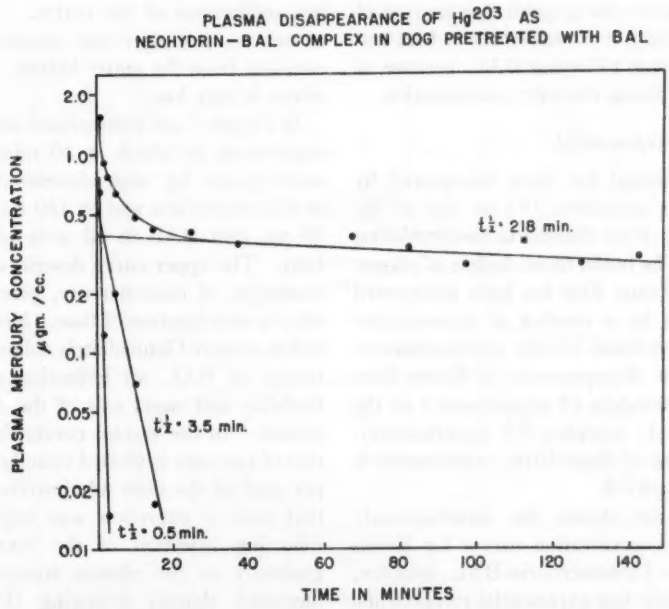


FIG. 3. AN ANALYSIS OF THE CURVE DESCRIBING THE REGRESSION OF PLASMA CONCENTRATION FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1.0 MG. OF MERCURY PER KG. AS THE CHLORMERODRIN-BAL COMPLEX TO A BAL TREATED DOG

merodrin complex in a BAL treated dog (13, 14). Since saline was administered intravenously at a rate of 5 ml. per minute for the duration of the experiment as well as for 90 minutes preceding the start of urine collection, the eventual increase of urine flow to 6 and 7 ml. per minute was not unexpected. However, not less than 96 per cent of the filtered sodium was reabsorbed during any period, a value incompatible with significant mercurial diuresis.

The clearance of mercury was very high during the first five minutes following the administration of the BAL-Chlormerodrin complex, *i.e.*, 89 ml. per minute in contrast to 0.8 ml. per minute in the experiment presented in Table I. For the first 30 minutes the clearance remained considerably higher than in the control experiment. However, except for the first 10 minutes, the absolute rate of excretion and hence the per cent of the dose excreted was lower in the BAL than in the control experiment. This ambiguity of high clearance, yet low rate of excretion finds explanation in the extremely low plasma concentrations of mercury in the BAL experiment. It is evident from these findings, namely slow excretion (only 20.6 per cent of the dose in 150 minutes) and low plasma concentration (roughly 1/10th of the values obtained in the initial periods of the experiment in Table I), that the half-time of the slow exponential must be increased, whereas that of either the intermediate or fast exponential or both must be reduced.

In Figure 3 is presented a graphic analysis of the experiment presented in Table II. It is evident that the slow exponential had a time course of the same order of magnitude as is observed after bilateral nephrectomy, a reflection of the very low rate of excretion of the BAL complex. In contrast, the intermediate exponential was definitely shortened, a function, we assume, of increased diffusibility. In this and in the 3 other experiments of a similar nature, the half-times of the intermediate exponentials have been reduced by roughly 50 per cent. In contrast the fast exponentials did not differ significantly from those observed in certain experiments with Chlormero-

drin alone. However, the graphic construction of this fast exponential is a bit uncertain at best and rather more uncertain following BAL, because of low and variable plasma mercury concentration.

Origin of the fast exponential

The fast exponential has been interpreted by Threefoot and his associates (1) as due to the mechanical mixing of the diuretic in the circulating plasma volume. The initial rapid decline of plasma concentration of Evans Blue has been interpreted in similar fashion by a number of investigators (15–17). We have made a series of simultaneous comparisons of the disappearance of Evans Blue and either Chlormerodrin (2 experiments) or the Chlormerodrin-BAL complex (4 experiments). An example of one of these latter experiments is summarized in Figure 4.

On the right are shown the simultaneously measured plasma concentration curves for Evans Blue and for the Chlormerodrin-BAL complex, and on the left, their fast exponential components. The range of uncertainty in determining half-times of these fast processes is large, ranging from 0.5 to 1.5 minutes. However, in this series fair agreement has been observed, a fact favoring the mixing hypothesis.

Renal extraction, excretion, and accumulation of Chlormerodrin

According to Greif, Sullivan, Jacobs, and Pitts (18), Chlormerodrin is accumulated in highest concentration in the cortex of the kidney of the dog, in lesser amounts in the outer medulla and scarcely at all in the papillary medulla. As a first approximation, it is reasonable to assume that the drug exerts its characteristic diuretic action where it is specifically and most highly concentrated.[8] Accordingly we have attempted to correlate diuresis with the accumulation of radiomercury in the renal cortex, measured qualitatively in terms of counts per minute by a Geiger tube in contact with the kidney surface. Because of the relatively low energy of the beta emission of Hg^{203} (0.205 MEV), beta activity measured at the surface is derived almost exclusively from the outermost few millimeters of the cortex. To some extent, of course, the Geiger tube responds to the gamma emission from the entire kidney, although its efficiency is very low.

In Figure 5 are summarized data obtained in an experiment in which at 30 minutes, 1.0 mg. of mercury per kg. was administered intravenously as Chlormerodrin and at 150 minutes, 2.0 ml. of 10 per cent BAL in oil were given intramuscularly. The upper curve describes the plasma concentration of radiomercury, plotted semilogarithmically as a function of time. Note the sharp drop which occurred immediately following the administration of BAL, an indication of increased diffusibility and rapid exit of the diuretic from the plasma. In the second curve, the urinary excretion of mercury is plotted cumulatively in terms of per cent of the dose administered. It is evident that rate of excretion was highest immediately following injection of the diuretic, diminished gradually as the plasma concentration fell and increased sharply following BAL. The third curve is that of radioactivity recorded at the surface of the kidney and represents qualitatively the accumulation of mercury in the outermost layers of the cortex. It is evident that accumulation was most rapid during the first 10 to 20 minutes following the diuretic, *i.e.*, during the time that the plasma concentration was highest and also falling most rapidly. Thereafter, accumulation proceeded more slowly, approaching a plateau of 2250 to

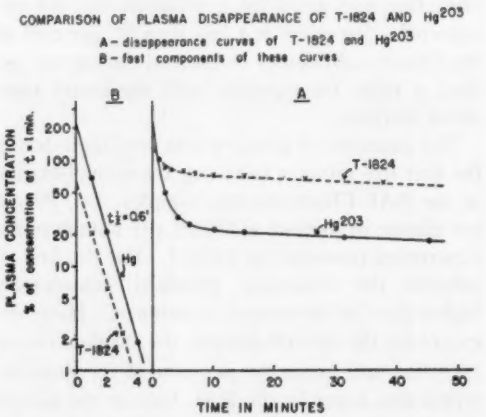


FIG. 4. A SIMULTANEOUS COMPARISON OF THE FAST COMPONENTS OF THE PLASMA REGRESSION CURVES FOR EVANS BLUE AND CHLORMERODRIN-BAL COMPLEX

[8] It is possible that accumulation in the renal cortex is concerned with renal tubular secretion, that secretion is unrelated to diuresis, and that diuresis results from a lesser accumulation in another part of the kidney.

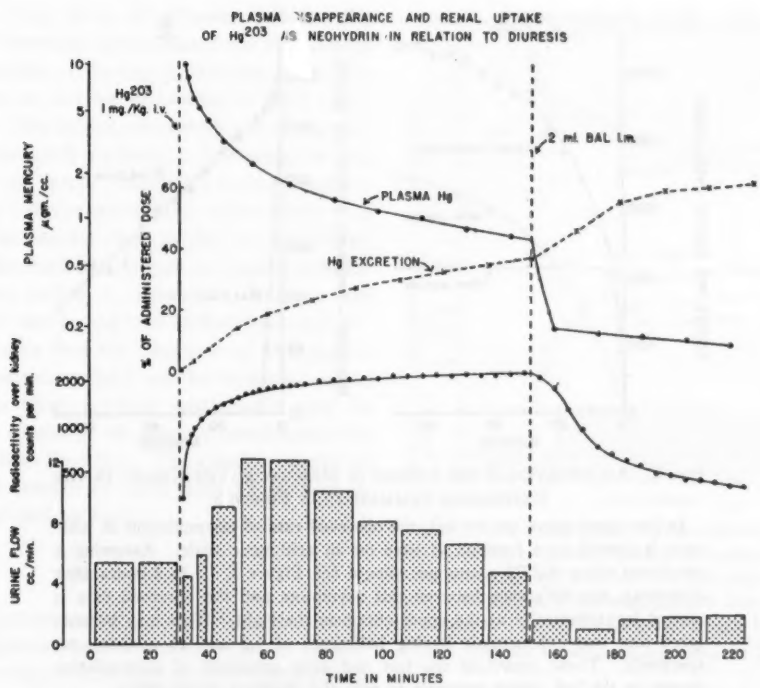


FIG. 5. AN EXPERIMENT ILLUSTRATING REGRESSION OF PLASMA CONCENTRATION OF MERCURY, EXCRETION OF MERCURY, ACCUMULATION OF MERCURY IN THE KIDNEY AND DIURESIS IN A DOG GIVEN 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN INTRAVENOUSLY

2300 counts per minute after an hour and a half. When BAL was given, the renal mercury was promptly discharged, in major part into the urine as shown by the excretion curve, in small part into the renal venous blood, as demonstrated in subsequent experiments. Urine flow, as shown at the bottom of the figure, dropped slightly in the first five minutes after the intravenous injection of Chlormerodrin, rose to a peak in 20 to 40 minutes and then diminished slowly over the subsequent hour and a half. With the administration of BAL, urine flow dropped sharply. It is evident that the onset of diuresis correlates well with the rapid uptake of mercury by the kidney during the first 30 minutes after the injection of the diuretic. The sharp drop in urine flow after BAL likewise correlates reasonably well with the loss of accumulated mercury, although it is known that increased secretion of Pitressin® contributes to the antidiuresis (19). However, it is evident that urine flow was not maintained at the peak diuretic value despite maintenance of a high level of accumulated mercury in the renal cortex. How well the diuresis is sustained depends in part on the magnitude of positive fluid and salt balance developed prior to administering the diuretic.

The accumulation of mercury by the kidney has been further analyzed in the manner shown in Figure 6. As is evident in the upper curve of Figure 6A, radioactivity, recorded over the kidney, increased rapidly for an hour or so after injection and then reached a plateau of roughly 2275 counts per minute, a fact apparent in Figure 5. In the upper curve of Figure 6B, the differences between this plateau and the observed counts are plotted semilogarithmically as a function of time. This curve, which expresses the rate at which the plateau is approached, may be analyzed graphically into two exponentials with half-times of 16.0 and 1.3 minutes, respectively. The two components of

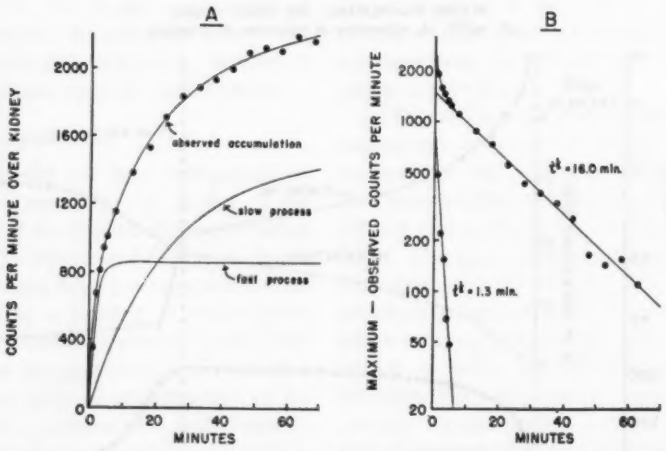


FIG. 6. AN ANALYSIS OF THE UPTAKE OF MERCURY BY THE KIDNEY IN THE EXPERIMENT SUMMARIZED IN FIGURE 5

In the upper curve on the left, the observed rate of accumulation of mercury is plotted as a function of time on an arithmetic scale. Assuming a maximum value of 2275 counts per minute (cf. Figure 5, 1.5 to 2 hours after injection), the difference between this asymptote and the observed rate is plotted logarithmically as the upper curve on the right. This may be analyzed into two exponentials having half-times of 1.3 and 16 minutes, respectively. These constitute the fast and slow processes of accumulation shown on the left, which summate to give the observed accumulation.

accumulation, one saturating rapidly, the other slowly, are shown as the lower two curves of Figure 6A. One might interpret these two exponentials in terms of two systems or mechanisms of binding. On the other hand only one mechanism might be operative and the two exponentials result from two components of plasma regression.

Renal extraction, excretion and accumulation of mercury were measured in absolute units in two dogs following the intravenous administration of 1.0 mg. of mercury per kg. as Chlormerodrin. These experiments are very similar in design to those of Weston and his co-workers (20) in man and may be considered as basically confirming their findings. One of the experiments, in which the animal was unilaterally nephrectomized immediately preceding the experiment, is summarized in Table III and Figure 7. Renal venous blood was obtained through a polyethylene catheter introduced through a jugular vein and positioned in the renal vein by manipulation through an abdominal incision. Extraction, excretion and accumulation are presented cumulatively in terms of micrograms of mercury. Renal plasma flow was calculated from rate of excretion and arterio-venous difference for creatinine, corrected for urine flow (2, 3). Extraction and accumulation of mercury by the kidney have been calculated as follows:

Renal Extraction (μgm./min.) = R.P.F.$\cdot$($A_{Hg} - R_{Hg}$) + V$\cdot R_{Hg}$.

Renal Accumulation (μgm./min.) = Renal extraction $- U_{Hg}\cdot V$.

R.P.F. = Renal plasma flow in ml./min.

A_{Hg} = Arterial plasma mercury concentration in μgm./ml.

R_{Hg} = Renal venous plasma mercury concentration in μgm./ml.

U_{Hg} = Urinary mercury concentration in μgm./ml.

V = Urine flow in ml./min.

It is evident from Table III and Figure 7 that mercury was much more rapidly extracted from the plasma than excreted during the interval immediately following injection of Chlormerodrin,

i.e., for the first 30 to 60 minutes. Hence the quantity of mercury accumulated in the kidney increased rapidly, a finding in the dog much like that of Weston and his associates in man (20). From 60 to 120 minutes, extraction and accumulation tended to reach a plateau. Following the administration of BAL, extraction became slightly negative, *i.e.*, the concentration of mercury was higher in the venous than in the arterial blood. However, the marked decrease in renal accumulation was due mainly to increased excretion and only in small part to negative extraction. It is interesting to note that the clearance of mercury in the two periods after BAL was far in excess of the renal plasma flow, a result explainable only in terms of the discharge of mercury accumulated in tubular cells into the urine. The quantity stored in the kidney decreased by more than 50 per cent in the course of half an hour.

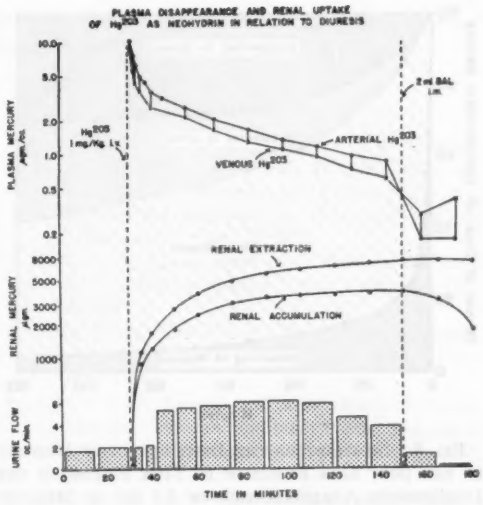


FIG. 7. AN EXPERIMENT ILLUSTRATING REGRESSION OF PLASMA CONCENTRATION, RENAL EXTRACTION AND RENAL ACCUMULATION OF MERCURY IN RELATION TO DIURESIS IN A UNILATERALLY NEPHRECTOMIZED DOG GIVEN 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN INTRAVENOUSLY

Data from the experiment presented in Table III.

DISCUSSION

Three major exponential rates determine the regression of plasma mercury concentration following the intravenous injection of Chlormerodrin in the dog. Similar observations were made following Meralluride in man (1). Each of these major rates is no doubt the resultant of the activity of a number of independent processes and only in a limited sense can one consider that any one process is the major determinant of any one rate.

The fast exponential with a half-time of 0.5 to 1.5 minutes would seem most clearly dominated by the physical process of mixing within the plasma compartment. The fact that we have observed good agreement between these components of the regression curves of Evans Blue and Chlormerodrin argues in favor of this hypothesis.

The intermediate exponential with a half-time of 6 to 9 minutes is obviously of complex origin. No doubt, as Threefoot and his co-workers (1) postulated, it represents diffusion of mercury into tissues and binding to tissue proteins. The kidney cortex binds mercury in highest concentration, namely 100 to 150 μgm. per gm.; liver and spleen bind the metal in lower concentration but in appreciable total amounts; skeletal muscle binds but little (11). Thus, penetration of a variety of tissues, perfused at different rates and exhibiting differing affinities for the heavy metal summate to yield the intermediate exponential of the plasma regression curve. BAL, which greatly increases the diffusibility of Chlormerodrin and increases the mercury content of all tissues and organs except the kidney, reduces the half-time of this intermediate exponential.

The slow exponential with a half-time of 50 to 80 minutes represents, in large part, activity of renal excretory mechanisms. This is most clearly shown by the fact that bilateral nephrectomy markedly decreases the rate of fall of plasma mercury from 30 minutes on. However, following nephrectomy, the slope of this portion of the plasma regression curve does not become zero. We assume that excretion into the gut and continuing penetration of tissues account for the residual slope of the slow component of the plasma regression curve.

The distribution of mercury in the body and its excretion in the urine following the intravenous administration of 1.0 mg. Hg per kg. as Chlormerodrin are summarized in Figure 8. The data upon which the graph is based were derived from the experiment presented in Table III and Figure 7. The per cent of the dose in the plasma compart-

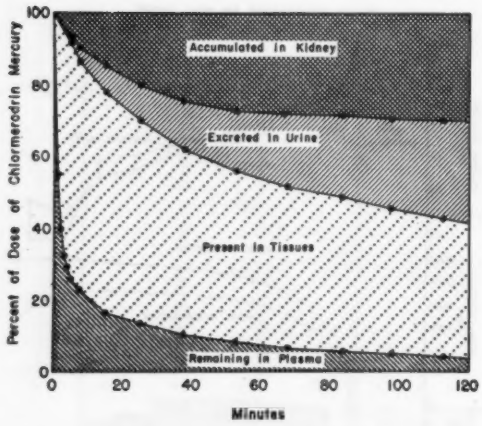


FIG. 8. SUMMARY OF THE DISTRIBUTION OF MERCURY IN THE BODY AS A FUNCTION OF TIME FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN

Data from the experiment presented in Table III and Figure 7.

ment was calculated as the product of the plasma concentration and the plasma volume, the latter estimated as 5 per cent of body weight. The per cent of the dose accumulated in the kidney and the per cent excreted in the urine were calculated as previously described. Since the animal was unilaterally nephrectomized, both excreted and accumulated moieties are no doubt less than would be found in the intact animal. The per cent taken up by the tissues was the difference between 100 and the sum of the three values for plasma content, kidney accumulation and excretion. This graph is rather inaccurate during the first few minutes but is reasonably valid thereafter. It resembles very closely one presented by Milnor, Burch, Ray, Threefoot, and Berenson (21), describing the distribution of Meralluride in man. Its significant features are the following: the mercury, initially delivered into the plasma, is rapidly distributed into the tissues, which thereafter serve as a reservoir to replenish the plasma store as renal accumulation and urinary excretion account for a progressively increasing fraction of the total.

In the past some have speculated that the mercury which accumulates in the tubular cells and serves to inhibit reabsorption, is that which has entered the glomerular filtrate. That this view is incorrect is evident from the data of Table III. The extraction of mercury from the renal arterial plasma averaged 22 per cent in this experiment. Since the filtration fraction averaged 0.23 and the filtrable moiety ranged between 1 and 5 per cent, it is obvious that not more than 1.5 per cent of the mercury delivered to the kidney in the arterial blood could have found its way into the filtrate. Therefore most and probably essentially all of the mercury must have entered the tubular cells from the peritubular fluid.

Although binding by plasma proteins restricts the entry of Chlormerodrin into the glomerular filtrate, it does not equally restrict distribution into tissues. A minute fraction of the compound is free and in equilibrium with the bound moiety. If tissue proteins, *e.g.*, renal tubular cells, have a higher affinity for Chlormerodrin than do the plasma proteins, the drug can be rapidly transferred from plasma to kidney, dissociating from plasma as taken up by kidney. The kinetics of this process in a variety of tissues, determines the time course of the intermediate exponential.

Tubular secretion of Chlormerodrin involves at least two processes, binding by and concentration in renal tubular cells and combination with some sulfhydril containing substance. According to Müller (6), essentially all of the excreted diuretic is eliminated in combination with either cysteine or acetyl cysteine. Binding might well be a *passive* process, depending on high affinity of cells of the renal cortex for the free valence of mercury in the mercurial. The *active* step in tubular secretion could then be assigned to the splitting of the cell complex and to the formation of the cysteine derivative. Transfer into tubular urine might depend on diffusion forces. This view is suggested by the observation that the administration of the sulfhydril compound, BAL, causes the rapid discharge of Chlormerodrin from the kidney, for the most part into the urine, but also into the renal venous blood. This last observation could mean that diffusion plays a role.

The action of Chlormerodrin as a diuretic must be related to its specific accumulation within renal tubular cells where it serves to block reversibly certain enzyme systems concerned with the reabsorption of salt. Delay in onset of diuresis following intravenous administration is no doubt re-

lated to the time required to accumulate some critical concentration of drug in the renal cortex. Cessation of diuretic activity is of course ultimately associated with complete elimination of drug in the urine. However, a maintained high renal concentration of the diuretic does not ensure a maintained high output of urinary salt and water. Magnitude of diuretic response depends in addition upon the volume of extracellular fluid, its ionic pattern and concentration, the adequacy of glomerular filtration and the degree of stimulation of tubular reabsorption (*22*).

SUMMARY

Following the intravenous injection in the dog of 1.0 mg. per kg. of Hg^{203} as Chlormerodrin, plasma concentration regresses as a multiple exponential function of time. Three exponentials have been identified with half-times of 0.5 to 1.5, 6 to 9 and 50 to 80 minutes, respectively. The first of these exponentials agrees reasonably well with that describing the initial regression of the plasma concentration of Evans Blue when the dye and diuretic are administered simultaneously. Thus, it must be dependent to a considerable degree on the physical process of mixing within the circulating volume of plasma. The second exponential represents an average of the rates of transfer of mercury from the plasma to a variety of tissues and organs perfused at varying rates and exhibiting different affinities for the diuretic. The administration of Chlormerodrin complexed with BAL greatly increases diffusibility of the mercury and its uptake by all tissues other than the kidney. Uptake by the kidney is reduced. The net effect of BAL is to speed significantly this second component of regression of plasma concentration. The third exponential is dominated by renal excretion as has been clearly demonstrated by bilateral nephrectomy. However, it represents to an appreciable degree extrarenal clearance, including gastro-intestinal secretion and a slow component of penetration of tissues.

Chlormerodrin is rapidly taken up by the renal cortex, fixed within the tubular cells, and excreted into the urine. Delay in the onset of diuresis following intravenous administration is related to the time required to accumulate a critical concentration of the drug within the tubular cells. Due to high plasma binding, most of the mercury excreted in the urine is eliminated by tubular secretion.

REFERENCES

1. Threefoot, S. A., Ray, C. T., Burch, G. E., Cronvich, J. A., Milnor, J. P., Overman, W., and Gordon, W., Concentration-time course in the plasma of man of radio-mercury introduced as a mercurial diuretic. J. Clin. Invest., 1949, **28**, 661.
2. Wolf, A. V., Total renal blood flow at any urine flow or extraction fraction. Am. J. Physiol., 1941, **133**, 496.
3. Smith, H. W., The Kidney: Structure and Function in Health and Disease. New York, Oxford Univ. Press, 1951.
4. Barrett, M. J., Chronic and acute effects of Mercuhydrin and Thiomerin on renal tubular function in the dog. J. Pharmacol. & Exper. Therap., 1950, **100**, 502.
5. Chinard, F. P., Estimation of plasma volume by dye dilution method *in* Methods in Medical Research, M. B. Visscher, Ed., Chicago, Year Book Publishers, 1951, vol. 4, p. 38.
6. Müller, O. H., Personal communication.
7. Burch, G., Ray, T., Threefoot, S., Kelly, F. J., and Svedberg, A., The urinary excretion and biological decay periods of radiomercury labeling a mercurial diuretic in normal and diseased man. J. Clin. Invest., 1950, **29**, 1131.
8. Grossman, J., Weston, R. E., Lehman, R. A., Halperin, J. P., Ullmann, T. D., and Leiter, L., Urinary and fecal excretion of mercury in man following administration of mercurial diuretics. J. Clin. Invest., 1951, **30**, 1208.
9. Milnor, J. P., Binding of the mercury of an organic mercurial diuretic by plasma proteins. Proc. Soc. Exper. Biol. & Med., 1950, **75**, 63.
10. Mulrow, P., and Fuller, G., Unpublished observations on plasma binding of Chlormerodrin.
11. Borghgraef, R. R. M., and Pitts, R. F., The distribution of Chlormerodrin (Neohydrin®) in tissues of the rat and dog. J. Clin. Invest., 1956, **35**, 31.
12. Parks, M. E., and Van Woert, W., Unpublished observations.
13. Handley, C. A., and La Forge, M., Effect of thiols on mercurial diuresis. Proc. Soc. Exper. Biol. & Med., 1947, **65**, 74.
14. Farah, A., and Maresh, G., The influence of sulfhydryl compounds on diuresis and renal and cardiac circulatory changes caused by mersalyl. J. Pharmacol. & Exper. Therap., 1948, **92**, 73.
15. Kennedy, J. A., and Millikan, G. A., A micro blood volume method using a blue dye and photocell. J. Physiol., 1938, **93**, 276.
16. Miller, A. T., Jr., A re-evaluation of the T-1824 mixing curve. Am. J. Physiol., 1947, **151**, 234.
17. Noble, R. P., and Gregerson, M. I., Blood volume in

clinical shock. I. Mixing time and disappearance rate of T-1854 in normal subjects and in patients in shock; determination of plasma volume in man from 10-minute sample. J. Clin. Invest., 1946, **25**, 158.

18. Greif, R. L., Sullivan, W. J., Jacobs, G. S., and Pitts, R. F., Distribution of radiomercury administered as labelled Chlormerodrin (Neohydrin®) in kidneys of rats and dogs. J. Clin. Invest., 1956, **35**, 38.
19. Earle, D. P., Jr., and Berliner, R. W., Effect of 2,3-dimercaptopropanol on diuresis. Am. J. Physiol., 1947, **151**, 215.
20. Weston, R. E., Grossman, J., Lehman, R. A., Ullmann, T. D., Halperin, J. P., and Leiter, L., Renal extraction and excretion of mercury in man following intravenously administered mercurial diuretics. J. Clin. Invest., 1951, **30**, 1221.
21. Milnor, P., Burch, G., Ray, T., Threefoot, S., and Berenson, G., Considerations of renal, hepatic and extremital arteriovenous differences in concentration of radiomercury of a mercurial diuretic. J. Clin. Invest., 1950, **29**, 72.
22. Pitts, R. F., and Sartorius, O. W., Mechanism of action and therapeutic use of diuretics. Pharmacol. Rev., 1950, **2**, 161.

take the pulse of your laboratory performance

A single physiological blood chemistry standard and control is now available to check reagents, equipment and technique. Versatol is the first standard in whole human serum, duplicating all the interfering substances in the patient's serum. The constituents for which Versatol is standardized are first removed from the serum . . . then replaced in known normal amounts. Thus you are assured of accurate and reproducible results.

Your laboratory can now perform the delicate PBI test without worrying about detecting contamination. Versatol is a control and standard for 12 laboratory determinations: serum protein-bound iodine . . . total nitrogen . . . total protein . . . nonprotein nitrogen . . . urea nitrogen . . . creatinine . . . sodium . . . potassium . . . chlorides . . . phosphates . . . calcium . . . glucose.

Easy to prepare: Versatol is reconstituted simply by adding distilled water. No extra, time-consuming steps are required. The solution can then be used like human serum.

Available from leading local laboratory supply distributors in boxes of ten 5-ml. vials, $21.00.

Versatol

Trademark

WARNER-CHILCOTT

Laboratory Supply Division, Morris Plains, New Jersey

REVUE FRANÇAISE D'ÉTUDES CLINIQUES ET BIOLOGIQUES

NOVEMBER, 1956

Editorial

ACTH, Cortisone and Experimental Infection. A. DELAUNAY.

Original Articles

Effect of X-Ray on Red Cell Fragility. O. LARTIGUE.

Use of the Mouse in the Bacteriologic Diagnosis of Tuberculosis. Application in the Rapid Evaluation of Drug Sensitivity. H. NOUFFLARD and S. BERTEAUX.

Measure of Thyroid Function by Radioactive Iodine (I^{131}). A. LOVERDO and R. FAUVERT.

"Zinc ACTH Test" in the Study of Adrenocortical Function. C. DARNAUD, Y. DENARD, G. MOREAU, A. SERRES, R. SARDOU, and P. VEREZ.

On Adrenocortical Function Among People of Different Age-Groups (Measure of Urinary 17-Ketosteroids, 17-Hydroxycorticoids, and Corticoids in Subjects 70 Years of Age Before, During and After Administration of ACTH, Cortisone, and Testosterone). L. BINET, E. E. BAULIEU, and M. F. JAYLE.

General Review

Hematopoiesis. A. GAJDOS.

Biochemistry of Melanosis and Melanuria. P. Gonnard.

Technical Note

Use of Radioactive Chromium in the Determination of the Total Blood Volume. A. LOVERDO.

Subscription, U.S.A. and Canada, $19.00

Special rate for interns and residents, $13.00

Flammarion, 1243 University Street, Montreal

FOR PROTEIN BOUND IODINE DETERMINATIONS

...The Hycel System

The PBI determination is the ONLY TEST which directly measures thyroid function by measuring the ability to produce circulating thyroxine.

Combining specially designed equipment, purified reagents and a simplified method, the Hycel System gives reproducible results to ±0.1 mcg%.

Equipment includes reagents for over 200 determinations and special equipment to perform up to ten tests daily. Also included are Manuals describing method and interpretation.

The Hycel System is the result of extensive research and testing by Hormone Chemistry Laboratory. They offer corroborative assays as a service to users.

No. 53845—Hycel PBI System.....**$340.00**

A multiple set is available containing sufficient reagents for over 600 tests, and additional equipment for performing 20 or more tests daily. Our catalog No. 53846, it sells for $1050.00.

Consult your SP representative for further details.

You can rely on Scientific Products—

- Ainsworth balances
- American Optical microscopes
- B.B.L. culture media
- Barnstead stills
- Bausch & Lomb microscopes
- Beck Lee E.K.G. equipment
- Beckman instruments
- Becton-Dickinson equipment
- Berkeley radioisotope equipment
- Browne-Morse sectional cabinetry
- Christian Becker balances
- Clay Adams medical equipment
- Coleman spectrophotometers
- Coors porcelain
- Corning glass
- Dade serums
- DiSPo items—disposable specialties
- Hartman-Leddon chemicals
- Hycel clinical testing equipment
- International centrifuges
- Jewett refrigerators
- Kimble glassware
- Labline laboratory equipment
- Lindberg hotplates & furnaces
- Lipshaw tissue equipment
- Mallinckrodt reagent chemicals
- Matheson Coleman & Bell organic chemicals
- Monaghan respiratory equipment
- Ohaus scales
- Precision scientific equipment
- Schieffelin pharmaceuticals
- SP laboratory specialties
- Spinco electrophoresis apparatus
- Sylvana serological reagents
- Torsion balances
- Virtis virus & tissue equipment
- Warner-Chilcott pharmaceuticals

No matter what your laboratory needs—be sure to consult your SP representative first!

SP Scientific Products

DIVISION OF AMERICAN HOSPITAL SUPPLY CORPORATION

New York • Chicago • Kansas City • Minneapolis • Atlanta • Washington • Dallas • Los Angeles • San Francisco

CLINICAL SCIENCE

Edited for the Medical Research Society by
W. MELVILLE ARNOTT

Assistant Editors
G. D. DAWSON, K. W. DONALD, H. E. DE WARDENER

Advisory Board

E. A. CARMICHAEL
R. V. CHRISTIE
R. T. GRANT
J. McMICHAEL
G. W. PICKERING
E. E. POCHIN
E. P. SHARPEY-SCHAFER
JANET VAUGHAN
W. C. WILSON
L. J. WITTS

Volume 15, No. 4. **Issued November, 1956.**

The Viscous Resistance of Lung Tissue in Patients with Pulmonary Disease. By ROBERT MARSHALL and ARTHUR B. DUBOIS.

Measurement of the Human Febrile Response to a Bacterial Pyrogen. By F. WENDT, E. S. SNELL, F. GOODALE, JR. and W. I. CRANSTON.

Inactivation of a Bacterial Pyrogen by Human Serum and Plasma. By F. GOODALE, JR., E. S. SNELL, F. WENDT and W. I. CRANSTON.

Observations on the Sickling Phenomenon and on the Distribution of Different Haemoglobin Types in Erythrocyte Populations. By A. C. ALLISON.

Isotope Investigations of Red Cell Production and Destruction in Chronic Renal Disease. By R. A. JOSKE, JOAN M. MCALISTER and T. A. J. PRANKERD.

Vascular Reactivity in Experimental Renal and Renoprival Hypertension. By E. G. MCQUEEN.

Pilomotor and Sudomotor Innervation in Diabetes. By FRANZ R. BARANY and EDWARD H. COOPER.

The Passage of Strontium Across the Intestinal Wall of the Rat. By H. G. JONES and C. R. COID.

Enterohepatic Circulation of Thyroxine in Humans. By N. B. MYANT.

The Estimation and Prediction of Normal Blood Volume. By D. A. HICKS, A. HOPE, A. L. TURNBULL and D. VEREL.

The Metabolism of a Therapeutic Dose of ^{45}Ca in a Case of Multiple Myeloma. By J. ANDERSON, E. W. EMERY, JOAN M. MCALISTER and S. B. OSBORN.

The Effect of Limb Position and of Venous Congestion on the Circulation Through the Toes. By R. M. ENGLAND and J. G. MCC. JOHNSTON.

A Comparison of the Effects of Acetylsalicylic Acid and Dl-triiodothyronine in Patients with Myxoedema. By W. D. ALEXANDER and K. W. M. JOHNSON.

Adrenocortical Response to Corticotrophin and Operation. By R. P. JEPSON, KATHLEEN M. EDWARDS and M. W. REECE.

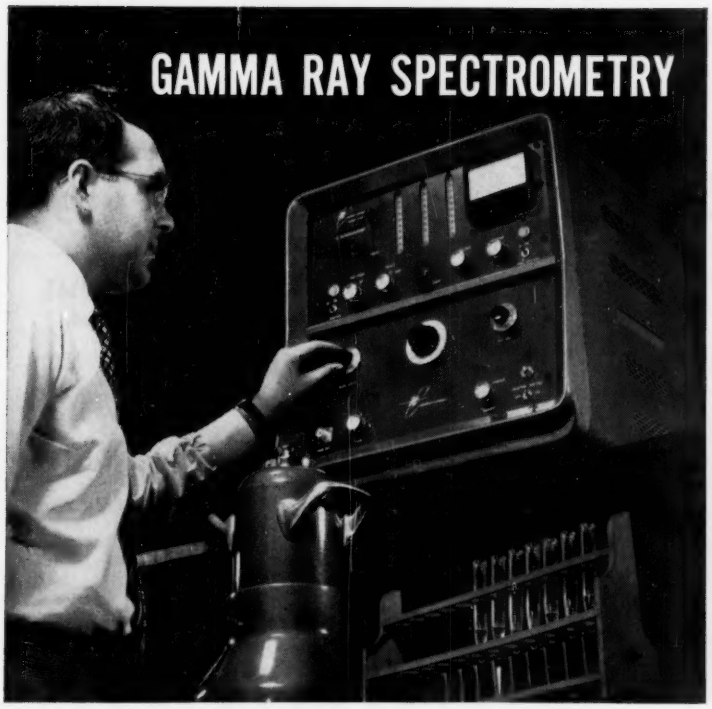
Response of the Blood Vessels of the Upper Extremity to Prolonged Local Heat. By AZIZ AHMAD.

Rapid Infusion and Renal Function. By HÄRJE BUCHT, JAN EK, BERTIL JOSEPHSON, BENGT THOMASSON, EDVARDAS VARNAUSKAS and LARS WERKÖ.

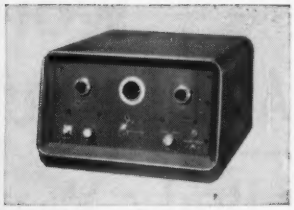
An Experimental Study of Magnesium Deficiency in Man. By M. G. FITZGERALD and PAUL FOURMAN.

Subscription Rate: £3.15. 0. per volume.

Publishers: Shaw & Sons Ltd., 7, 8 & 9, Fetter Lane, Fleet Street, London, E. C. 4, England



Gamma emitting samples inserted into a scintillation well counter are counted by Radiation Analyzer and standard scaler. Adjustable "window" of Analyzer is set to count only pulses that fall within a pre-selected portion of the energy spectrum, thus virtually eliminating "background".



Analyzer is available separately as an economical accessory for laboratories already having a scintillation detector and a scaler or rate-meter.

Automatic scanning and recording of spectrum energies is accomplished by Analyzer, rate-meter, recorder and scintillation counter. Gamma energy spectrum of Cesium-137 is shown on recorder chart.

...with *nuclear-chicago's*

NEW RADIATION ANALYZER

and your *present* scaler and scintillation counter

The advantages of pulse-height selection for background and scatter-error reduction, multiple tagging measurement and energy analysis are now available for every radioisotope laboratory. The Radiation Analyzer, connected directly to your present scaler and scintillation counter, incorporates in a compact single-chassis design a non-overloading linear amplifier, pulse height discriminator and extremely stable high voltage regulator. Circuitry has been designed for simplicity, stability and high reliability.

SPECIFICATIONS

- Linear feedback amplifier with gain of 2500.
- Non-overload amplifier with delay-line shaper. Separate amplifier output.
- Base level adjustable 0-100 volts with ten-turn potentiometer. Channel width of 0-10 volts provided by precision single turn control. Computer tubes, precision components and ultra-stable power supplies provide long term stability.
- Regulator for scaler high voltage supply variable from 500 to 1500 volts with ten-turn potentiometer. Regulation factor of 200.
- Overall resolution time for equal pulse pairs within 1.5 microsecond.
- Front panel switch provides integral or differential count.
- Provision for external base level control allows automatic scanning.

nuclear-chicago

Write today for full details

NUCLEAR INSTRUMENT AND CHEMICAL CORPORATION

269 West Erie Street, Chicago 10, Illinois

LEADERS IN MAKING RADIOACTIVITY COUNT

APPARATUS & EQUIPMENT SECTION

Body Water Determinations

- Raw serum—no distillation
- 30-minute test results
- 1% precision
- Inexpensive tests, and equipment
- Safe, convenient

Advanced Tritium Analyzer

ADVANCED INSTRUMENTS, INC. 17 Oakcrest Road Needham, Mass.

CARDIAC CATHETERIZATION

Laboratories throughout theworld use equipment specifically designed for them by

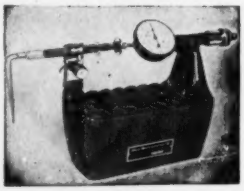
ELECTRONICS for MEDICINE

17 So. Lexington Ave.

White Plains, N. Y.

DIRECT-DIAL READING VOLUME DELIVERY FROM 1 TO 100 μL

SYRINGE MICROBURET

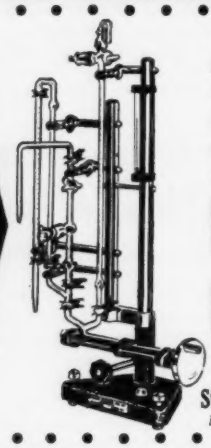


Simple, fast, accurate deliveries. Interchangeable syringes give equivalent of a series of microburets, speeds multiple analysis on large numbers of samples.

Write today for CATALOG!

MICRO-METRIC INSTRUMENT CO.

P. O. BOX 884, CLEVELAND 22, OHIO



The NATELSON MICROGASOMETER *Can Also Be Used As A* MICRO BURETTE *With Unusually Fine Ease of Control!*

Simple Analysis of Blood for Gas Content Using Only 0.03 ml.

PLUS

Converts into accurate Micro Burette 0 to 0.12 ml. by .001 ml.

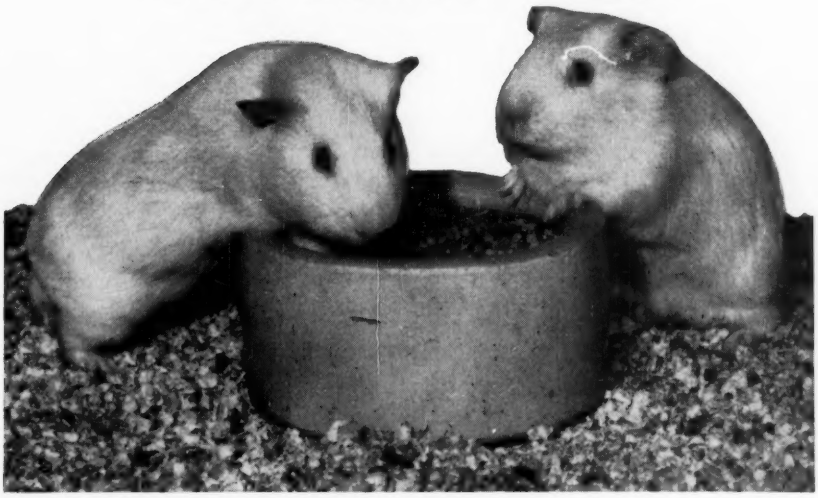
For information see your laboratory supply dealer or write to:

SCIENTIFIC INDUSTRIES, Inc.

15 Park St., Springfield, Mass.

Purina *laboratory animal* Nutrition News

NOW, A COMPLETE CHOW FOR GUINEA PIGS



Purina Research has just released a new complete feed for guinea pigs, requiring no ascorbic acid or vegetables as a supplement. Purina Guinea Pig Chow contains all the nutrients guinea pigs need for good reproduction, lactation, growth and health. It is made in 5/32", Bite-Size Checker form.

This is an important step forward in the feeding of laboratory guinea pigs. It has always been a nuisance to provide the essential Vitamin C or ascorbic acid requirements of guinea pigs by feeding vegetables containing this vitamin or by feeding it through the drinking water.

The Vitamin C used in Purina's new Guinea Pig Chow has been stabilized to hold its potency more than 30 days after it reaches your laboratory. In ordering Guinea Pig Chow, you can estimate your needs by figuring the amount needed per animal at 1½ to 3 lbs. per month, depending on age.

This new, easy-to-feed, complete Guinea Pig Chow can be ordered through any one of the more than 5,000 Purina Dealers, located from coast to coast.

For further information about feeding laboratory guinea pigs and for the analysis of Guinea Pig Chow, write to Mr. Albert H. Leonard, Special Industry Sales, Ralston Purina Company, St. Louis 2, Mo.

RALSTON PURINA COMPANY, St. Louis 2, Missouri

With Over 5,000 Dealers Coast to Coast To Serve You

A new use for

Schwarz®

N-Ethyl Maleimide (NEM)

for specific
detection of
SH compounds
and
thiol esters

•

simple
sensitive

•

It is now possible to detect and distinguish sulfhydryl compounds by a color reaction with NEM. The reagent is particularly useful for developing paper chromatograms. Details of the procedure appear in Science 123, 981 (June 1, 1956).

Among Schwarz products giving positive color reaction with NEM are:

Glutathione
S-Acetyl Glutathione
DL-Homocysteine Thiolactone • HCL
***N*-Acetyl DL-Homocysteine Thiolactone**
L-Cysteine • HCl • H_2O
Cysteinyl Glycine
Thiouracil

Write for further information

SCHWARZ LABORATORIES, INC.

Leading Manufacturers of Sulfhydryl Reagents and Yeast Biochemicals

230 WASHINGTON STREET, MOUNT VERNON, NEW YORK SL 370C

LANCASTER PRESS, INC., LANCASTER, PA.